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(54) Title: NOVEL CARD PROTEINS INVOLVED IN CELL DEATH REGULATION

(57) Abstract: The present invention provides NB-ARC and CARD-containing proteins (NACs), nucleic acid molecules encoding NACs and antibodies specific for at least one NAC. The invention further provides chimeric NAC proteins. The invention also provides screening assays for identifying an agent that can effectively alter the association of an NAC with an NAC-associated protein. The invention further provides methods of modulating apoptosis in a cell by introducing into the cell a nucleic acid molecule encoding an NAC or an antisense nucleotide sequence. The invention also provides a method of using a reagent that can specifically bind to anUS0024125iagnose a pathology that is characterized by an increased or decreased level of apoptosis in a cell.

NOVEL CARD PROTEINS INVOLVED IN CELL DEATH REGULATION

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the fields of molecular biology and molecular medicine and more specifically to the identification of proteins involved in programmed cell death and associations of these proteins.

BACKGROUND INFORMATION

Programmed cell death is a physiologic process that ensures homeostasis is maintained between cell production and cell turnover in essentially all self-renewing tissues. In many cases, characteristic morphological changes, termed "apoptosis," occur in a dying cell. Since similar changes occur in different types of dying cells, cell death appears to proceed through a common pathway in different cell types.

In addition to maintaining tissue homeostasis, apoptosis also occurs in response to a variety of external stimuli, including growth factor deprivation, alterations in calcium levels, free-radicals, cytotoxic lymphokines, infection by some viruses, radiation and most chemotherapeutic agents. Thus, apoptosis is an inducible event that likely is subject to similar mechanisms of regulation as occur, for example, in a metabolic pathway. In this regard, dysregulation of apoptosis also can occur and is observed, for example, in some types of cancer cells, which survive for a longer

time than corresponding normal cells, and in neurodegenerative diseases where neurons die prematurely. In viral infections, induction of apoptosis can figure prominently in the pathophysiology of the disease process, because immune-based eradication of viral infections depends on elimination of virus-producing host cells by immune cell attack resulting in apoptosis.

Some of the proteins involved in programmed cell death have been identified and associations among some of these proteins have been described. However, additional apoptosis regulating proteins remain to be found and the mechanisms by which these proteins mediate their activity remains to be elucidated. The identification of the proteins involved in cell death and an understanding of the associations between these proteins can provide a means for manipulating the process of apoptosis in a cell and, therefore, selectively regulating the relative lifespan of a cell or its relative resistance to cell death stimuli.

The principal effectors of apoptosis are a family of intracellular proteases known as Caspases, representing an abbreviation for Cysteine Aspartyl Proteases. Caspases are found as inactive zymogens in essentially all animal cells. During apoptosis, the caspases are activated by proteolytic processing at specific aspartic acid residues, resulting in the production of subunits that assemble into an active protease typically consisting of a heterotetramer containing two large and two small subunits (Thornberry and Lazebnik, Science 281:1312-1316 (1998)). The phenomenon of apoptosis is produced directly or indirectly by the activation of caspases in cells, resulting in the proteolytic cleavage of specific substrate proteins. Moreover, in many cases,

caspases can cleave and activate themselves and each other, creating cascades of protease activation and mechanisms for "auto"-activation.

Among the substrates of caspases are the intracellular proforms of cytokines such as pro-Interleukin-1β (pro-IL-1β) and pro-IL-18. When cleaved by caspases, these pro-proteins are converted to the biologically active cytokines which are then liberated from cells, circulating in the body and eliciting inflammatory immune reactions. Thus, caspases can be involved, in some instances, in cytokine activation and responses to infectious agents, as well as inflammatory and autoimmune diseases. Caspases also participate in signal transduction pathways activated by some cytokine receptors, particularly members of the Tumor Necrosis Factor (TNF) family of cytokine receptors which are capable of activating certain caspase zymogens.

Thus, knowledge about the proteins having domains that interact with and regulate caspases is important for devising strategies for manipulating cell life and death in therapeutically useful ways. The identification of such proteins that contain caspase-interacting domains and the elucidation of the proteins with which they interact, therefore, can form the basis for strategies designed to modulate apoptosis, cytokine production, cytokine receptor signaling, and other cellular processes. Thus a need exists to identify proteins that interact with caspases and other apoptosis related proteins. The present invention satisfies this need and provides additional advantages as well.

SUMMARY OF THE INVENTION

In accordance with the present invention, there are provided novel "NB-ARC and CARD"-containing proteins, designated NAC, as well as several isoforms of NAC produced by alternative mRNA splicing. The invention also provides nucleic acid molecules encoding NAC and its isoforms, vectors containing these nucleic acid molecules and host cells containing the vectors. The invention also provides antibodies that can specifically bind to NAC proteins, including alternative isoforms thereof.

The present invention also provides a screening assay useful for identifying agents that can effectively alter the association of NAC with itself or with other proteins. By altering the self-association of NAC or by altering their interactions with other proteins, an effective agent may increase or decrease the level of caspase proteolytic activity or apoptosis in a cell, or it may increase or decrease the levels of NF-κB, cytokine production, or other events.

The invention also provides methods of altering the activity of NAC in a cell, wherein such increased or decreased activity of NAC can modulate the level of apoptosis or other cellular responses. For example, the activity of NAC in a cell can be increased by introducing into the cell and expressing a nucleic acid sequence encoding these proteins. In addition, the activity of NAC in a cell can be decreased by introducing into the cell and expressing a fragment of NAC, or an antisense nucleotide sequence that is complementary to a portion of a nucleic acid molecule encoding the NAC proteins.

The invention also provides methods for using an agent that can specifically bind NAC or a nucleotide sequence that can bind to a nucleic acid molecule encoding NAC to diagnose a pathology that is characterized by an altered level of apoptosis due to an increased or decreased level of NAC in a cell.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A shows a schematic representation showing the domain structure of the longest isoform of human NAC, referred to as NACβ. The NB (Nucleotide Binding) domain (amino acids 329-547, filled box), the leucine-rich repeats (LRR, amino acids 808-947, filled bars), and the CARD (Caspase-Associated Recruitment Domain) (amino acids 1373-1473, dotted box) are depicted. Hatched boxes indicate sequences derived from two alternatively spliced exons.

Figure 1B shows the amino acid sequence of the longest human NAC isoform (also set forth in SEQ ID NO:2). The positions for the P-loop (Walker A) and Walker B of NB-domain are indicated. The amino acids sequences of LRR repeats and CARD are underlined and in bold letters, respectively. Italic letters indicate sequences for the alternatively spliced exons.

Figure 1C shows a sequence analysis of NAC: NB-ARC homology. Alignment of the NB-domain of NAC (amino acids 329-547) to the NB-domain of Nod1/CARD4 (amino acids 197-408), and the NB-ARC domains of Apaf-1 (amino acids 138-355) and *C. elegans* CED-4 (amino acids 154-374). Alignment was conducted using the Clustal W. method (Thompson et al., Nuc. Acids Res. 22:4673-4680 (1994)). Identical and similar residues are shown in black and

gray shades, respectively. Positions of P-loop and Walker B sequences are indicated.

Figure 1D shows the alignment of CARD domain of NAC (amino acids 1373-1465), Nod1/CARD4 (amino acids 15-104), Apaf-1 (amino acids 1-89), and CED-4 (amino acids 2-89). Identical and similar residues are shown in black and gray shades, respectively.

Figure 1E shows the 3D-structure prediction of the NAC CARD domain. The structure of the CARD domain of NAC was modeled based on the structures of Raidd, Apaf-1, and pro-caspase-9. Six α-helixes are labeled (H1 through H6). Models of the predicted structure of the CARD domain of NAC were generated using the MODPELLER program, essentially as described (Schendel et al. (1999) *JBC* 274, 21932-21936), based on the structures of the CARDs of Apaf-1, pro-caspase-9, and Raidd (Chou et al. (1998) *Cell* 94, 171-180; and Qin et al.(1999) *Nature* 399(6736), 549-557).

Figure 2 shows multiple isoforms of NAC. Isoforms of NAC are generated by alternative mRNA splicing, based on cDNA cloning results. The same symbols as in Figure 1A are used. Two alternatively spliced exons are shown as dotted boxes and hatched boxes, respectively. The four resultant isoforms are described as NACα, NACβ, NACγ and NACδ.

Figure 3 shows the results from Example 4.0 demonstrating the ATP-dependent self-association of NB-domain. (A) Purified GST control protein or GST fusion protein containing the NB-domain of NAC (GST-NB) (0.5 μg

immobilized on GSH-Sepharose beads) were incubated with ^{35}S-labeled, in vitro translated (IVT) NB-domain of NAC (NAC-NB) (top) or Pro-Caspase-9 (bottom). Bound proteins were eluted and analyzed by SDS-PAGE. One-tenth of input IVT proteins (lane 1) were directly loaded for comparison. (B) IVT[^{35}S]-NAC-NB in reticulocyte lysates was incubated at 30°C for 30 min with (+) or without (-) various concentrations (0-10 units) of apyrase (Sigma) to deplete ATP prior to absorption to GST or GST-NB as indicated. (C) Binding of IVT[^{35}S]-NAC-NB to immobilized GST-NB was performed in the presence of various concentrations of γ-S-ATP. (D) Mutation in the P-loop of NAC NB-domain prevents self-association. Wild-type (WT) (*lanes 1-3*) and K340M mutant (*lanes 4-6*) NB-domains of NAC were produced as GST-fusions and purified from bacteria, or they were produced as ^{35}S-labeled proteins by in vitro translation (IVT). GST control or GST fusions containing NB-domain of either WT or K340M immobilized on glutathione-Sepharose (0.5 μg) were incubated with 1 ml of IVT'd proteins as above, then washed, and adsorbed proteins were eluted and analyzed by SDS-PAGE. One-tenth of input IVT proteins (lanes 1 and 4) was included for comparison.

Figure 4 shows the results from Example 5.0 demonstrating that NAC forms complexes with itself and Ced-4 family proteins. (A) CARD-CARD interactions. IVT[^{35}S]-labeled CARD domain of NAC, CARD of Apaf-1, CED-4, or pro-caspase-9 and Bcl-10 were incubated with immobilized GST (lane 2) or GST fusion containing the CARD of NAC (NAC-CARD) (lane 3). One-tenth of input IVT[^{35}S] proteins were also loaded directly into gels (lane 1). (B) Full-length NAC forms complexes with Apaf-1, CED-4, and itself. Human 293T cells were cultured in 6-well plates and transiently transfected with expression plasmids (pcDNA3,

1 μg each) encoding epitope-tagged (HA or Flag tag) full-length HA-NAC, full-length Flag-Apaf-1, HA-Apaf-1 lacking the WD repeats [HA-Apaf-1(ΔWD)], HA-CED-4, or an inactive form of pro-caspase-9 harboring a catalytic site mutation (C287A) (HA-Caspase-9) in the presence (+) or absence (-) of pcDNA3 (1 μg) encoding myc-tagged full-length NAC (myc-NAC). Immunoprecipitations (IP) were performed 1 day later using either a mouse monoclonal antibody to myc (lanes 4 and 6) or a control mouse IgG (Cntl) (lane 5). Immune-complexes were resolved by SDS-PAGE and analyzed by immunoblotting using anti-HA or anti-Flag antibodies. Lysates derived from each transfection (10% of IP input) were loaded directly in gels as controls (lanes 1-3). Alternatively, these interaction results were further confirmed by probing myc-NAC in immune complexes derived from the indicated target proteins. (C) NAC associates with Nod1. Myc-tagged full-length NAC was co-transfected with or without Flag-tagged Nod1. Immunocoprecipitation was performed using anti-Flag M2 monoclonal antibodies and the resultant immune-complexes were probed for NAC using anti-myc antibody (lanes 3 and 4). Lysates derived form each transfection (10 % of IP input) were directly loaded in gels as controls (lanes 1 and 2). Alternatively, NAC/Nod1 interaction was further confirmed by probing Nod1 in NAC immune-complexes. (D) Gel-sieve chromatography analysis of Apaf-1/NAC protein complexes. Lysates prepared from cells co-expressing Flag-tagged Apaf-1 and myc-tagged NAC were treated with cyt-c (10 μM)/dATP (1 μM) for 5 min. at 30°C, then 100 mm zVAD-fmk was added and the samples were placed on ice before fractionation on a Superose-6 gel-filtration column. Column fractions were assayed by SDS-PAGE/immunobloting for Apaf-1 and NAC, using anti-Flag and anti-myc epitope antibodies, respectively. The positions of molecular weight markers and the void volume fraction are

indicated. Fractions containing Apaf-1 and NAC were pooled, immunoprecipitated with anti-Flag antibodies to recover Apaf-1 or with an IgG control antibody (Cntl), and the resulting immune-complexes were immunoblotted for NAC using anti-myc epitope antibody. (E) Time-course of NAC/Apaf-1 association induced by cyt-c. Cell lysates expressing Flag-Apaf-1 and HA-NAC were treated with cyt-c (10 μM)/dATP (1 mM) for various times and subjected to immunoprecipitation with anti-Flag antibodies. The resulting Apaf-1 immuno-complexes were analyzed by immunobloting for the presence of NAC. Lane 6 (asterisk) shows results when the assay was performed in the presence of a caspase inhibitor, zVAD-fmk (50 μM). Note that caspase inhibitor appeared to stablize the Apaf-1/NAC complex.

Figure 5 shows the results from Example 7.0 demonstrating that NAC modulates cyt-c-induced caspase activation. (A) NAC enhances cyt-c-induced pro-caspase-9 processing and DEVD cleavage activity. Human 293T cells were transfected with pcDNA3 plasmid (10 μg) encoding full-length NAC or empty vector (CNTL) in 10 cm plates. Cytoplasmic extracts were prepared from transfected cells after 24 h, using hypotonic, detergent-free conditions. Cell lysates (10 μg) were incubated with [^{35}S] pro-Caspase-9 in the presence or absence of cyt-c (10 μM) and dATP (1 μM) (cyt-c) at 30°C for 60 min. To monitor pro-Caspase-9 processing, reaction mixtures were resolved on SDS-PAGE and visualized by fluorography (upper panel). Alternatively, DEVDase activity in extracts was measured. Various concentrations of cyt-c were added (lower panel) and release of AFC from the caspase substrate Ac-DEVD-AFC was monitored [expressed as relative fluorescent units (RFU) per mg protein per minute]. (B) Human 293T cells were transfected with pcDNA3 (10 μg) containing NAC cDNA

in reverse orientation (NAC AS) or empty vector (CNTL) in 10 cm plates. Cytoplasmic extracts were prepared after 36 h culture, normalized for protein concentration, and assayed for cyt-c activation of caspases (DEVDases). Results are expressed as percent of control (mean ± SE, n=3). Upper panel shows an immunoblot of NAC and Apaf-1 in cell lysates derived from parallel experiments by co-expressing epitope-tagged NAC or Apaf-1 with or without AS-NAC, showing antisense-mediated reductions in NAC but not Apaf-1. (C). DEVDase activity was measured in cell lysates following GST affinity-adsorption performed using GST control or GST-fusion proteins containing the CARD or NB-domain (NB) of NAC. DEVDase activity induced by cyt-c (1 μM) and dATP (1 mM) was measured continuously, monitoring AFC release from Ac-DEVD-AFC (RFU/μg protein). Insert represents Coomassie stained SDS-PAGE gel analysis of GST fusion proteins used for the adsorptions. (D) Cell extracts were subjected to GST-affinity absorption as in "C" and then caspases were activated using either 10 ng GraB or 1 μM cyt-c (mean % GST control ± SE, n=3).

Figure 6 shows the results from Example 8.0 demonstrating that NAC enhances Apaf-1 induction of apoptosis and caspase activation. (A) 293T cells were transfected with pEGFP (0.1 μg) and plasmids encoding pro-Caspase-9 (0.05 μg), Apaf-1 (0.05 or 2.0 μg) or NAC (0.5, 1, or 2 μg), as indicated. Total DNA input was normalized with empty vector. Transfected cells were cultured in media containing 0.1% fetal bovine serum for 1.5 days, and then fixed and stained with DAPI. The % GFP-positive apoptotic cells (exhibiting nuclear fragmentation and chromatin condensation) was determined by fluorescence microscopy (mean ± SE, n=3). (B) Cytoplasmic extracts of 293T cells transfected with plasmids encoding pro-Caspase-9 (0.05 μg DNA), Apaf-1 (0.05 μg), NAC (2 μg) or

various combinations as indicated were assayed for caspase activity by addition of AC-DEVD-AFC to cell lysates and continuous monitoring of AFC release (RFU/ µg lysate) over time. Though not shown, negligible caspase activity was detected in lysates of cells transfected with Apaf-1, NAC, or the combination of NAC and Apaf-1 (in the absence of pro-caspase-9). (C) Triple complex formation involving NAC, Apaf-1, and caspase-9. 293T cells were transfected with Flag-tagged Apaf-1 in combination with Flag-tagged pro-caspase-9 (lanes 2 and 3) and myc-tagged NAC (lane 3). Transfected cells were cultured in the presence of zVAD-fmk (100 µM) for 24 h, then lysed under hypotonic, detergent-free conditions in the presence of 50 µM zVAD-fmk. Cell lysates were subjected to immunoprecipitation using a rabbit polyclonal anti-human caspase-9 antibody. The resulting immune complexes were fractionated on SDS-PAGE, transferred to nitrocellulose membranes, and subsequentially probed for the presence of transfected Apaf-1 and pro-caspase-9 using anti-Flag antibody (*upper panel*), and for NAC using anti-myc antibody (*lower panel*). (D) 293T cells were transfected with 0.1 µg pEGFP DNA and either 0.5 or 2 µg of plasmids encoding the CARD or NB-domain of NAC, together with expression plasmids encoding pro-caspase-9 (0.05 µg) and Apaf-1 (1 µg), Fas (0.3 µg). Alternatively, cells were treated with Staurosporine (STS, 1 µM for 5 h). Cells were fixed and stained with DAPI and scored for apoptosis (mean ± SE, n=3).

Figure 7 shows the results from Example 9.0 demonstrating that NAC enhances Nod1 induction of apoptosis. (A) 293T cells were transfected with pEGFP (0.1 µg) and various amounts of expression plasmids encoding pro-caspase-9 (0.05 µg), Nod1, NAC, and NAC-CARD as indicated (total

DNA input for each transfection was normalized with empty vector). Transfected cells were cultured in media containing 0.1% fetal bovine serum for 1.5 days, and then fixed and stained with DAPI. The % GFP-positive apoptotic cells (exhibiting nuclear fragmentation and chromatin condensation) was determined by fluorescence microscopy (mean ± SE, n=3). (B) NAC can indirectly associate with caspase-9 through Nod1. 293T cells were transiently transfected with expression plasmids encoding an inactive form of pro-caspase-9 harboring a replacement mutation of the catalytic residue C287A [pro-caspase-9 (C287A)] (1 μg) together with Flag-tagged Nod1 (1 μg) and myc-tagged NAC (1 μg), as indicated. Total DNA input for each transfection was normalized with empty vector. Cytoplasmic extracts were prepared using buffer A and subjected to immunoprecipitation (IP) with rabbit polyclonal anti-caspase-9 antibodies (lanes 4-6). The resulting immune-complexes were resolved by SDS-PAGE, transferred to nitrocellulose membrane, and sequentially probed for NAC, Nod1, and caspase-9 using specific antibodies. One tenth of lysates used for IP from each transfection were directly loaded on gels for controls (Lysate; lanes 1-3).

Figure 8 shows effect of CARD-X on Bax-mediated apoptosis as set forth in Example 11.0.

Figure 9 shows the effect of CARD-X on caspase9-mediated apoptosis as set forth in Example 12.0.

Figure 10 shows the results from Example 13.0 demostrating that CARD-X competes with Apaf-1 for binding Caspase-9.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided "substantially pure" mammalian CARD-containing proteins, designated NAC and CARD-X. As used herein, the term "NAC" refers to a protein that contains both an NB-ARC domain and a CARD domain (NAC). The invention NAC proteins represent novel members of the "CARD domain" family of proteins, which family includes CED-4 and Apaf-1. An invention NAC comprises a NB-ARC domain and a CARD domain, and optionally further comprises a leucine-rich repeat domain and/or a TIM-Barrel-like domain.

An exemplary invention NAC is a large, multi-domain protein, containing a CARD, NB-domain, and LRRs. The CARD domain of NAC is capable of associating with the CARD-containing members of the CED-4-family, including CED-4, Apaf-1, and Nod1, but does not appear to interact with CARD-carrying caspases or other CARD-containing proteins tested. Thus, NAC represents a new type of apoptosis regulator heretofore undescribed, which interacts specifically with CED-4-family proteins. In this regard, the Apaf-1 and CED-4 proteins directly bind CARD-containing caspases, and promote protease activation upon oligomerization by bringing the sub-optimally-active pro-enzymes into close proximity, allowing them to trans-process each other. In contrast, it has been found NAC enhances Cyt-c-mediated processing of pro-caspase-9, but it does not directly bind pro-caspase-9 (nor caspases-1, 2, 6, 7, 8, 10, or 11). Rather, interaction of NAC with Apaf-1 facilitates Apaf-1-mediated activation of pro-caspase-9, thus revealing a new paradigm for regulation of Apaf-1/CED-4 family proteins.

It is intriguing that cells are known to vary in their sensitivity to Cyt-c-induced activation of caspases in ways that cannot be accounted for by differences in the levels of Apaf-1 protein. This observation thus implies variations in the efficiency with which Apaf-1 can induce processing and activation of pro-caspase-9. The discovery of NAC and the evidence presented herein that it is capable of associating with Apaf-1 and modulating Apaf-1-dependent caspase activation indicates a mechanism for fine-tune signaling through the Cyt-c/Apaf-1 apoptosis pathway -- namely, by altering the levels of NAC protein.

It has been proposed that the CED-4-family member, Nod1, may provide an alternative Apaf-1-independent mechanism in cells for activating pro-caspase-9. It has been found that Nod1 associates with pro-caspase-9 and induces apoptosis, at least when over-expressed in cells. Similar to observations described herein regarding Apaf-1, it has been found that NAC associates with Nod1 and that it appears to exist in a complex together with Nod1 and pro-caspase-9. These findings indicate that NAC also indirectly modulates the activation of pro-caspase-9 by Nod1, akin to its effects on Apaf-1. In contrast to Apaf-1, however, little is known thus far about the physiological roles of Nod1 in vivo. While Nod1 contains LRRs, representing candidate protein interactions domains that may link Nod1 activation to an upstream pathway (analogous to the interaction of the WD domains of Apaf-1 with Cyt-c), the identity of that pathway remains unknown. Similarly, it is contemplasted herein that the LRRs in NAC also mediate binding to unidentified proteins, thereby providing additional mechanisms for coupling certain cell death or cell survival stimuli to core components of the apoptosis machinery through

interactions with NAC, which in turn binds and enhances the activities of CED-4-family proteins.

The mechanism by which NAC enhances caspase-activation and apoptosis-induction by Apaf-1 and Nod1 may include one of the following. First, the CARD domain of NAC mediates interactions with CED-4 family proteins, presumably through CARD-CARD interactions. However, the CARDs of Apaf-1 and Nod1 also mediate their interactions with pro-caspase-9 via their N-terminal CARD-containing prodomain. Because co-immunoprecipitation experiments described herein indicate that NAC can interact with complexes of Apaf-1/pro-caspase-9 and complexes of Nod1/pro-caspase-9, the skilled artisan could expect that the CARDs of Apaf-1 and Nod1 can simultaneously bind the CARDs of NAC and pro-caspase-9 through hetero-multimerization as opposed to dimerization of CARDs.

Second, it has been found herein that the NB-domain of NAC is capable of self-associating in an ATP-dependent manner, analogous to the NB-domains of CED-4-family proteins. Also as provided herein, over-expression of the NB-domain of NAC in cells interfered with apoptosis induction by Apaf-1 and Nod1, indicating a trans-dominant inhibitory effect and further indicating that oligomerization of NAC molecules via this domain is important to its function. It has also been found that the NB-domain of NAC also suppressed apoptosis induced by staurosporine, a drug that induces apoptosis through the mitochondrial (Cyt-c/Apaf-1) pathway, but did not inhibit apoptosis induced by Fas, a prototypical death receptor which kills via a parallel pathway in the cells ("Type-I") used herein. It is contemplated herein that the NB-domain of NAC facilitates assembly of large multi-protein complexes ("apoptosomes") containing Apaf-1 or Nod1, along with relevant caspases, and possibly other

molecules. Molecular-sieve chromatography analysis of an invention NAC indicates that it resides in very large protein complexes, at least when over-expressed in cells, consistent with the results described herein.

Third, the interaction of NAC with Apaf-1 is affected by the activation state of Apaf-1, with Cyt-c-activated Apaf-1 displaying greater affinity for NAC than inactive Apaf-1. Thus, conformational changes induced in Apaf-1 by Cyt-c-binding may expose domains that are necessary for association with NAC. Analogously, it is contemplated herein that interactions of NAC with Nod1 are similarly subject to regulation in cells and NAC exists in inactive versus active conformations.

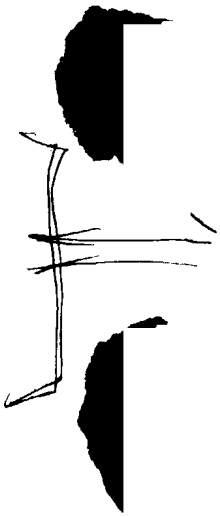
Fourth, although reconstitution of the Apaf-1/pro-caspase-9 apoptosome in vitro using purified components has demonstrated that NAC is not an essential cofactor, it is contemplated herein that invention NACs nevertheless play a contributory role in vivo, acting to (a) enhance assembly of the apoptosome, (b) slow disassembly of the apoptosome; or (c) enhance the catalytic activity of the Apaf-1/caspase-9 holoenzyme complex. Accordingly, methods of modulating the assembly or disassembly of apoptosome formation, or the catalytic activity of the holoenzyme complex are contemplated herein by contacting cells or NAC with an agent that modulates such assembly, or catalytic activity.

NAC is similar to other CED-4 family members in that it contains a CARD and NB-domain. For example, NAC possesses a NB-domain (also referred to herein as an NB-ARC domain) that mediates self-oligomerization, analogous to the NB-domains of CED-4 and Apaf-1. Further, the CARD

domain of NAC, while not interacting with the CARDs of caspases, does associate with other CARDs. These similarities, together with functional evidence that NAC is capable of modulating the activity of CED-4 family proteins, suggest that invention NACs represent members of a potentially large family of apoptosis-regulating proteins which combine apoptosis protein interaction domains (such as CARDs) with self-oligomerizing NB-domains, for the purpose of regulating multi-protein assemblies involved in caspase activation. Accordingly, by analogy to the ATP-binding pockets of protein kinases, it is contemplated herein to target the NB-domains of this class of apoptosis regulators using small-molecule drugs, thereby arriving at new therapeutics for diseases where apoptosis plays a role. Thus, invention NACs, or functional fragments thereof (e.g., NB- or CARD-domains) are useful in methods of identifying agents (e.g., small molecules) that bind to either the NB-domain or CARD-domain of NAC, and that modulate the aopoptosis mediating activity of NAC.

As used herein, the term "CARD domain" refers to a Caspase Recruitment Domain (Hofmann et al., Trends Biochem. Sci. 22:155-156 (1997)). CARD domains have been found in some members of the Caspase family of cell death proteases. Caspases-1, 2, 4, 5, 9, and 11 contain CARD domains near their NH_2-termini. These CARD domains mediate interactions of the zymogen inactive forms of caspases with other proteins which can either activate or inhibit the activation of these enzymes. For example, the CARD domain of pro-caspase-9 binds to the CARD domain of a caspase-activating protein called Apaf-1 (Apoptosis Protease Activating Factor-1). Similarly, the CARD domain of pro-caspase-1 permits interactions with another

CARD protein known as Cardiac (also referred to as RIP2 and RICK), which results in activation of the caspase-1 protease (Thome et al., Curr. Biol. 16:885-888 (1998)). And, pro-caspase-2 binds to the CARD protein Raidd (also know as Cradd), which permits recruitment of pro-caspase-2 to Tumor Necrosis Factor (TNF) Receptor complexes and which results in activation of the caspase-2 protease (Ahmad et al., Cancer Res. 57:615-619 (1997)). CARD domains can also participate in homotypic interactions with themselves, resulting in self-association of proteins that contain these protein-interaction domains and producing dimeric or possibly even oligomeric complexes.



CARD domains can be found in association with other types of functional domains within a single polypeptide, thus providing a mechanism for bringing a functional domain into close proximity or contact with a target protein via CARD:CARD associations involving two CARD-containing proteins. For example, the *Caenorhabiditis elegans* cell death gene ced-4 encodes a protein that contains a CARD domain and a ATP-binding oligomerization domain called an NB-ARC domain (van der Biezen and Jones Curr Biol 8:R226-R227). The CARD domain of the CED-4 protein interacts with the CARD domain of a pro-caspase called CED-3. The NB-ARC domain allows CED-4 to self-associate, thereby forming an oligomeric complex which brings associated pro-CED-3 molecules into close proximity to each other. Because most pro-caspases possess at least a small amount of protease activity even in their unprocessed form, the assembly of a complex that brings the proforms of caspase into juxtaposition can result in trans-processing of zymogens, producing the proteolytically processed and active caspase. Thus, CED-4 employs a CARD domain for binding a pro-caspase and

an NB-ARC domain for self-oligomerization, resulting in caspase clustering, proteolytic processing and activation.

Numerous CED-4-related proteins have recently been identified. These proteins belong to the CED-4 family of proteins, and include CED-4 (Yuan and Horvitz, Development 116:309-320 (1992)), Apaf-1, (Zou et al., Cell 90:405-413 (1997)), Dark (Rodriguez et al., Nature Cell Biol. 1:272-279 (1999)), and CARD4/Nod1 (Bertin et al., J. Biol. Chem. 274:12955-12958 (1999) and Inohara et al., J. Biol. Chem. 274:14560-14567 (1999)). As used herein, a CED-4 family member is a protein that comprises a NB-ARC domain and a CARD domain.

The CED-4 homolog in humans and rodents, referred to as Apaf-1, has been found to function similarly. The Apaf-1 protein contains a (i) CARD domain, (ii) NB-ARC domain, and (iii) multiple copies of a WD-repeat domain. In contrast to CED-4 which can spontaneously oligomerize, the mammalian Apaf-1 protein is an inactive monomer until induced to oligomerize by binding of a co-factor protein, cytochrome c (Li et al., Cell 91:479-489 (1997)). In Apaf-1, the WD repeat domains prevent oligomerization of the Apaf-1 protein, until coming into contact with cytochrome c. Thus, the WD-repeats function as a negative-regulatory domain that maintains Apaf-1 in a latent state until cytochrome c release from damaged mitochondria triggers the assembly of an oligomeric Apaf-1 complex (Saleh, J. Biol. Chem. 274:17941-17945 (1999)). By binding pro-caspase-9 through its CARD domain, Apaf-1 oligomeric complexes are thought to bring the zymogen forms of caspase-9 into close proximity, permitting them to cleave each other and produce the

proteolytic processed and active caspase-9 protease (Zou et al., J. Biol. Chem. 274:11549-11556 (1999)).

In addition to their role in caspase-activation, CARD domains have been implicated in other cellular processes. Some CARD-containing proteins, for example, induce activation of the transcription factor NF-κB. NF-κB activation is induced by many cytokines and plays an important role in cytokine receptor signal transduction mechanisms (DiDonato et al., Nature 388:548-554 (1997)). Moreover, CARD domains are found in some proteins that inhibit rather than activate caspases, such as the IAP (Inhibitor of Apoptosis Protein) family members, cIAP1 and cIAP2 (Rothe et al., Cell 83:1243-1252 (1995)) and oncogenic mutants of the Bcl-10 protein (Willis et al., Cell 96:35-45 (1999)). Also, though caspase activation resulting from CARD domain interactions is often involved in inducing apoptosis, other caspases are primarily involved in proteolytic processing and activation of inflammatory cytokines (such as pro-IL-1β and pro-IL-18). Thus, CARD-containing proteins can also be involved in cytokine production, thus regulating immune and inflammatory responses.

In view of the function of the CARD domain within invention NAC proteins, invention NAC proteins or CARD-domain containing fragments thereof, are useful herein in methods to modulate apoptosis, cytokine production, cytokine receptor signaling, and other cellular processes. Invention NAC proteins or CARD-domain containing fragments thereof are also useful herein to identify CARD-binding agents that modulate apoptosis, cytokine production, cytokine receptor signaling, and other cellular processes.

In one embodiment, a CARD domain of an invention NAC comprises a sequence with at least 50% identity to the CARD domain of NAC (see, e.g., residues 1373-1473 of SEQ ID NO:2). More preferably, a CARD domain of the invention comprises a sequence with at least 60% identity to the CARD domain of NAC. Most preferably, a CARD domain of the invention comprises a sequence with at least 75% identity to the CARD domain of NAC. Typically, a CARD domain of the invention comprises a sequence with at least 95% identity to the CARD domain of NAC.

As described herein, invention NAC or CARD-X proteins can associate with other CARD-containing proteins. In particular, the association of the CARD domain of invention NAC or CARD-X proteins with other CARD-containing proteins, such as Apaf-1, CED-4, caspases-1, 2, 9, 11, cIAPs-1 and 2, CARDIAK, Raidd, Dark, CARD4, and other NAC or CARD-X, and the like (also referred to herein as NAPs or CAPs, is sufficiently specific such that the bound complex can form *in vivo* in a cell or *in vitro* under suitable conditions. Similarly therefore, an invention NAC protein can associate with another NAC protein by CARD:CARD association.

A NAC or CARD-X protein of the invention further can associate, either directly or indirectly, with pro-caspases, caspases (e.g., Caspase-9) or with caspase-associated proteins, thereby modulating caspase proteolytic activity (see, e.g., Example 10.0 to 13.0 herein). Caspase proteolytic activity is associated with apoptosis of cells, and additionally with cytokine production. Therefore, an invention NAC or CARD-X can modulate apoptosis or cytokine production by modulating caspase proteolytic activity. As used herein a "caspase" is any member of the cysteine aspartyl proteases that

associates with a NAC protein of the invention or with a NAC associated protein. Similarly, a "pro-caspase" is an inactive or less-active precursor form of a caspase, which is typically converted to the more active caspase form by a proteolytic event.

CARD-containing proteins are also known to induce activation of the transcription factor NF-κB. Thus, an invention NAC can also modulate transcription by modulation of NF-κB activity.

A NAC protein of the invention also comprises a NB-ARC domain. As described herein, a NB-ARC domain of the invention NAC protein comprises a sequence wherein the identity of residues in either the P-Loop (Walker A) or Walker B regions is at least 60% relative to the residues of NAC (see, e.g., residues 329-342 and 406-414 of SEQ ID NO:2; see Figure 1B). Preferably, an NB-ARC domain of the invention NAC comprises a sequence wherein the overall identity of residues in the P-Loop (Walker A) and Walker B regions is at least 60% relative to the residues of NAC. More preferably, an NB-ARC domain of the invention comprises a sequence with at least 60% identity to the entire NB-ARC domain of NAC (see, e.g., residues 329-547 of SEQ ID NO:2). Most preferably, an NB-ARC domain of the invention comprises a sequence with at least 80% identity to the entire NB-ARC domain of NAC.

The NB-ARC domain of the invention NAC proteins associates with other proteins, particularly with proteins comprising NB-ARC domains. Thus, a functional NB-ARC domain associates with NB-ARC domain-containing proteins by way of NB-ARC:NB-ARC association. As used herein, the term "associate" or "association" means that NAC can bind to a protein relatively specifically and,

therefore, can form a bound complex. In particular, the association of the NB-ARC domain of NAC with another NB-ARC domain-containing proteins is sufficiently specific such that the bound complex can form *in vivo* in a cell or *in vitro* under suitable condition. Further, a NB-ARC domain demonstrates both nucleotide-binding (e.g., ATP-binding) and hydrolysis activities, which is typically required for its ability to associate with NB-ARC domain-containing proteins. Thus, an NB-ARC domain of the invention NAC comprises one or more nucleotide binding sites. As used herein, a nucleotide binding site is a portion of a protein that specifically binds a nucleotide such as, e.g., ATP, and the like. Typically, the nucleotide binding site of NB-ARC will comprise a P-loop, a kinase 2 motif, or a kinase 3a motif of the invention NAC (these motifs are defined, for example, in van der Biezen and Jones, supra). Preferably, the nucleotide binding site of NB-ARC comprises a P-loop of the invention NAC.

An invention NAC, therefore, is capable of CARD:CARD association and/or NB-ARC:NB-ARC association, resulting in a multifunctional protein capable of one or more specific associations with other proteins. An invention NAC can modulate cell processes such as apoptosis, cytokine production, and the like. For example, it is contemplated herein that an invention NAC protein can increase the level of apoptosis in a cell. It is also contemplated herein that an invention NAC can decrease the level of apoptosis in a cell. For example, a NAC which does not induce apoptosis may form hetero-oligomers with a NAC which is apoptotic, thus interfering with the apoptosis-inducing activity of NAC.

In another embodiment of the invention the NAC protein of the invention also contains Leucine-Rich Repeats (LRR) domain, similar to a LRR described in another CARD protein known as CARD4 (also known as Nod1) (Inohara et al., J. Biol. Chem. 274:14560-14567 (1999)). Unlike CARD-4 (Nod1), however, the CARD domain of NAC is located at the Carboxyl end of the protein whereas the CARD domain of CARD-4 (Nod1) is found at the NH_2-end of the protein. The function of the LRR domain is to mediate specific interactions with other proteins.

As used herein, leucine-rich repeat (LRR) domain of the invention NAC comprises a sequence with at least 50% identity to the LRR domain of NAC (see, e.g., residues 808-947 of SEQ ID NO:2). Preferably, a LRR domain of the invention NAC comprises a sequence with at least 60% identity to the LRR domain of NAC. More preferably, a LRR region of the invention NAC comprises a sequence with at least 75% identity to the LRR domain of NAC. Most preferably, a LRR region of the invention NAC comprises a sequence with at least 95% identity to the LRR domain of NAC.

It is further contemplated herein that a shortened LRR of the invention NAC may be used. A shortened LRR of the invention comprises a sequence with at least 90% identity to the splice variant form of the LRR (see, e.g., residues 808-917 of SEQ ID NO:2), and does not contain more than 90% of the residues in the splice region (see, e.g., residues 918-947 of SEQ ID NO:2). Preferably, the shortened LRR does not contain more than 70% of the residues in the splice region. More preferably, the shortened LRR does not contain more than 50% of the residues in the splice region. The shortened LRR will be of particular utility when the

protein:protein interaction activity of a NAC comprising a shortened LRR differs from that observed for a NAC comprising the full-length LRR. Activity of a NAC with a shortened LRR will be determined by one or more of the assays disclosed herein, and shall be considered to differ from that of a NAC comprising the full-length LRR if any protein:protein interactions are altered by 10% or more, or if caspase activity or apoptotic activity is altered by 10% or more.

In a further embodiment of the invention, invention NAC proteins contain a TIM-Barrel-like domain with similarity to TIM-barrel proteins. TIM-Barrel domains are well known in the art and typically consist of eight alternating α-helices and β-strands forming a barrel-like structure, but may contain 7 α-helices and/or β-strands in some instances. TIM-barrels have been found in some enzymes, such as aldolase, but also mediate protein interactions in some instances.

As used herein, a TIM-Barrel-like domain of an invention NAC comprises a sequence with at least 50% identity to the TIM-Barrel-like domain of NAC (residues 1079-1320 of SEQ ID NO:2). Preferably, a TIM-barrel-like domain of the invention NAC comprises a sequence with at least 60% identity to the TIM-Barrel-like domain of NAC. More preferably, a TIM-barrel domain of the invention NAC comprises a sequence with at least 70% identity to the TIM-barrel-like domain of NAC. Most preferably, a TIM-barrel-like domain of the invention NAC comprises a sequence with at least 80% identity to the TIM-barrel-like domain of NAC.

Presently preferred NAC proteins of the invention include proteins that comprise substantially the same

amino acid sequences as the protein sequence set forth in SEQ ID NOs:2, 4, and 6, as well as biologically active, functional fragments thereof.

Those of skill in the art will recognize that numerous residues of the above-described sequences can be substituted with other, chemically, sterically and/or electronically similar residues without substantially altering the biological activity of the resulting NAC protein species. In addition, larger polypeptide sequences containing substantially the same sequence as amino acids set forth in SEQ ID NOs:2, 4, and 6, therein are contemplated.

As employed herein, the term "substantially the same amino acid sequence" refers to amino acid sequences having at least about 70% identity with respect to the reference amino acid sequence, and retaining comparable functional and biological activity characteristic of the protein defined by the reference amino acid sequence. Preferably, proteins having "substantially the same amino acid sequence" will have at least about 80%, more preferably 90% amino acid identity with respect to the reference amino acid sequence; with greater than about 95% amino acid sequence identity being especially preferred. It is recognized, however, that polypeptides (or nucleic acids referred to hereinbefore) containing less than the described levels of sequence identity arising as splice variants or that are modified by conservative amino acid substitutions, or by substitution of degenerate codons are also encompassed within the scope of the present invention.

The term "biologically active" or "functional", when used herein as a modifier of invention NACs, or

polypeptide fragments thereof, refers to a polypeptide that exhibits functional characteristics similar to a NAC. Biological activities of NAC are, for example, the ability to bind, preferably *in vivo*, to a CARD-containing protein or a NB-ARC-containing protein, or to homo-oligomerize, or to modulate protease activation, particularly caspase activation, or to modulate NF-κB activity, or to modulate apoptosis, as described herein. Such NAC binding activity can be assayed, for example, using the methods described herein. Another biological activity of NAC is the ability to act as an immunogen for the production of polyclonal and monoclonal antibodies that bind specifically to an invention NAC. Thus, an invention nucleic acid encoding NAC will encode a polypeptide specifically recognized by an antibody that also specifically recognizes a NAC protein (preferably human) including the amino acid set forth in SEQ ID NOs:2, 4, 6, 10 or 12. Such immunologic activity may be assayed by any method known to those of skill in the art. For example, a test-polypeptide encoded by a NAC cDNA can be used to produce antibodies, which are then assayed for their ability to bind to an invention NAC protein including the sequence set forth in SEQ ID NOs:2, 4, 6, 10 or 12. If the antibody binds to the test-polypeptide and the protein including the sequence encoded by SEQ ID NOs:2, 4, 6, 10 or 12 with substantially the same affinity, then the polypeptide possesses the requisite immunologic biological activity.

As used herein, the term "substantially purified" means a protein that is in a form that is relatively free from contaminating lipids, proteins, nucleic acids or other cellular material normally associated with a protein in a cell. A substantially purified NAC can be obtained by a variety of methods well-known in the art,

e.g., recombinant expression systems described herein, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography, and the like. Other well-known methods are described in Deutscher et al., Guide to Protein Purification: Methods in Enzymology Vol. 182, (Academic Press, (1990)), which is incorporated herein by reference. Alternatively, the isolated polypeptides of the present invention can be obtained using well-known recombinant methods as described, for example, in Sambrook et al., supra., (1989).

In addition to the ability of invention NAC proteins, or fragements thereof, to interact with other, heterologous proteins (i.e., NB-ARC and CARD-containing proteins), invention NAC and CARD-X proteins have the ability to self-associate. This self-association is possible through interactions between CARD domains, and also through interactions between NB-ARC domains. Further, self-association can take place as a result of interactions between LRR and TIM-Barrel-like domains.

In accordance with the invention, there are also provided mutations and fragments of NAC which have activity different than a wild type NAC activity. As used herein, a "mutation" can be any deletion, insertion, or change of one or more amino acids in the wild type protein sequence, and a "fragment" is any truncated form, either carboxy-terminal, amino-terminal, or both, of the wild type protein. Preferably, the different activity of the mutation or fragment is a result of the mutant protein or fragment maintaining some but not all of the activities of wild type NAC. For example, a fragment of NAC can contain a CARD domain and LRR and TIM-Barrel-like domains, but lack a functional NB-ARC domain. Such a

fragment will maintain a portion of the wild type NAC activity (e.g., CARD domain functionality), but not all wild type activities (e.g., lacking an active NB-ARC domain). The resultant fragment will therefore have activity different than wild type NAC activity. In one embodiment, the activity of the fragment will be "dominant negative." A dominant negative activity will allow the fragment to reduce or inactivate the activity of one or more isoforms of wild type NAC.

Isoforms of the NAC proteins are also provided which arise from alternative mRNA splicing and may alter or modify the interactions of the NAC protein with other proteins. For example, three novel isoforms of NAC are provided herein and designated: NACβ, NACγ and NACδ (set forth as SEQ ID Nos:1, 3 and 5, respectively). The amino acid sequence and the portion of cDNA encoding the amino acid sequence of NACβ is shown in Figure 1B, and the NACβ cDNA and amino acid sequences are listed as SEQ ID NOs: 1 and 2, respectively. NACβ represents the NAC splice variant in which both splice regions are present in the translated polypeptide, thereby including the nucleic acids 1-4422 of the NAC cDNA sequence and amino acids 1-1473 of the NAC protein sequence of Figure 1B. NACγ represents the NAC splice variant in which neither splice region is present in the translated polypeptide, thereby including the nucleic acids 1-2868, 2962-3780, and 3916-4422 of the NAC cDNA sequence and amino acids 1-956, 998-1260, and 1306-1473 of the NAC protein sequence of Figure 1B. The NACγ cDNA and amino acid sequences are listed as SEQ ID NOs:3 and 4, respectively. NACδ represents the NAC splice variant in which only the more carboxy-terminal splice region is present in the translated polypeptide, thereby including the nucleic

acids 1-2868, and 2962-4422 of the NAC cDNA sequence and amino acids 1-956, and 998-1473 of the NAC protein sequence of Figure 1B. The NACδ cDNA and amino acid sequences are listed as SEQ ID NOs:5 and 6, respectively.

In another embodiment of the invention, chimeric proteins are provided comprising NAC, or a functional fragment thereof, fused with another protein or functional fragment thereof. Functional fragments of NAC include, for example, NB-ARC, CARD, LRR and TIM-Barrel-like domains, as defined herein. Proteins with which the NAC or functional fragment thereof are fused will include, for example, glutathione-S-transferase, an antibody, or other proteins or functional fragments thereof which facilitate recovery of the chimera. Further proteins with which the NAC or functional fragment thereof are fused will include, for example, luciferase, green fluorescent protein, an antibody, or other proteins or functional fragments thereof which facilitate identification of the chimera. Still further proteins with which the NAC or functional fragment thereof are fused will include, for example, the LexA DNA binding domain, ricin, α-sarcin, an antibody, or other proteins which have therapeutic properties or other biological activity.

Further invention chimeric proteins contemplated herein are chimeric proteins wherein a domain of the NAC is replaced by a similar such domain from a heterologous protein. For example, the NB-ARC domain of NAC, as described above, can be replaced by the NB-ARC domain of Apaf-1, and the like. Another example of such a chimera is a protein wherein the CARD domain of NAC is replaced by the CARD domain from CED-4, and the like.

The CARD-X protein contains a CARD domain and a region with similarity to TIM-Barrel-like domains, but otherwise is distinct from NAC. The cDNA sequence encoding CARD-X (SEQ ID NO:7) reveals that it arises from a separate gene from NAC. The predicted CARD-X amino acid sequence (SEQ ID NO:8), in particular, does not contain an NB-ARC domain.

A CARD domain of the CARD-X protein comprises a sequence with at least 50% identity to the CARD domain of CARD-X (residues 343-431 of SEQ ID NO:8). More preferably, a CARD domain of the invention comprises a sequence with at least 60% identity to the CARD domain of CARD-X. Most preferably, a CARD domain of the invention comprises a sequence with at least 75% identity to the CARD domain of CARD-X. Typically, a CARD domain of the invention comprises a sequence with at least 95% identity to the CARD domain of CARD-X.

A TIM-Barrel-like domain of CARD-X comprises a sequence with at least 50% identity to the TIM-Barrel domain of CARD-X (residues 56-331 of SEQ ID NO:8). Preferably, a TIM-barrel domain of the invention NAC comprises a sequence with at least 60% identity to the TIM-Barrel domain of CARD-X. More preferably, a TIM-barrel domain of the invention CARD-X comprises a sequence with at least 70% identity to the TIM-barrel domain of CARD-X. Most preferably, a TIM-barrel domain of the CARD-X comprises a sequence with at least 80% identity to the TIM-barrel domain of CARD-X.

In one embodiment, invention chimeric CARD-containing proteins provided herein are designated NAC-X. Nucleic acids that encode NAC-X are also provided herein. Alternative isoforms of the NAC-X proteins and the corresponding nucleic acids that encode the

alternative isoforms are also provided. As used herein, the term "NAC-X" refers to chimeric proteins comprising portions of a NAC and portions of CARD-X. For example, one type of NAC-X protein is a NACδ-X, wherein a portion of NACδ, for example, the TIM-Barrel-like domain of NACδ, is replaced by a portion of CARD-X, for example, the TIM-Barrel-like domain of CARD-X. It is within the scope of this invention that a protein comprising portions of a domain common to both NAC and CARD-X, particularly the CARD and TIM-Barrel-like domains, can comprise a chimera of NAC and CARD-X. For example, a NACβ-X protein can have residues 1-1397 from SEQ ID NO:2 immediately followed by residues 364-402 from SEQ ID NO:8, which are in turn immediately followed by residues 1436-1473 from SEQ ID NO:2, thus forming a chimeric CARD domain.

In one embodiment, a NAC-X protein will comprise an NB-ARC domain of NAC, as previously described, and the CARD domain of CARD-X. In another embodiment, a NAC-X protein will comprise the NB-ARC domain and LRR domain of NAC, the CARD domain of CARD-X, and the TIM-Barrel-like domain from either NAC or CARD-X or a chimera from both. In yet another embodiment, NAC-X will comprise the NB-ARC and LRR domains of NAC and the CARD and TIM-Barrel-like domains of CARD-X. For example, invention chimeric proteins can include residues between 1-947 and 1-1078 of NACβ (SEQ ID NO:2) or between 1-918 and 1-1048 of NACγ or NACδ (SEQ ID NOs:4 and 6, respectively) in chimera with residues between 1-431 and 56-431 of CARD-X (SEQ ID NO:8). A particular invention chimera is termed NAC-X1 a protein, and comprises the following sequences: NACβ-X1, residues 1-1078 of NACβ and residues 56-431 of CARD-X, having the resultant amino acid sequence listed in SEQ ID NO:10; NACγ/δ-X1 residues 1-1048 of NACγ or NACδ and

residues 56-431 of CARD-X, having the resultant amino acid sequence listed in SEQ ID NO:12. The cDNA encoding NACβ-X1 comprises cDNA residues 1-3234 of NACβ and 166-1293 of CARD-X, having the resultant sequence listed in SEQ ID NO:9; and the cDNA encoding NACγ/δ-X1 proteins comprise cDNA residues 1-3144 of NACγ or NACδ and 166-1293 of CARD-X, having the resultant sequence listed in SEQ ID NO:11.

Another embodiment of the invention provides NAC, or a functional fragment thereof, fused with a moiety to form a conjugate. As used herein, a "moiety" can be a physical, chemical or biological entity which contributes functionality to NAC or a functional fragment thereof. Functionalities contributed by a moiety include therapeutic or other biological activity, or the ability to facilitate identification or recovery of NAC. Therefore, a moiety will include molecules known in the art to be useful for detection of the conjugate by, for example, by fluorescence, magnetic imaging, detection of radioactive emission. A moiety may also be useful for recovery of the conjugate, for example a His tag or other known tags used for protein isolation/purification, or a physical substance such as a bead. A moiety can be a therapeutic compound, for example, a cytotoxic drug which can be useful to effect a biological change in cells to which the conjugate localizes.

An example of the means for preparing the invention polypeptide(s) is to express nucleic acids encoding the NAC in a suitable host cell, such as a bacterial cell, a yeast cell, an amphibian cell (i.e., oocyte), or a mammalian cell, using methods well known in the art, and recovering the expressed polypeptide, again using

well-known methods. Invention polypeptides can be isolated directly from cells that have been transformed with expression vectors as described below herein. The invention polypeptide, biologically functional fragments, and functional equivalents thereof can also be produced by chemical synthesis. For example, synthetic polypeptides can be produced using Applied Biosystems, Inc. Model 430A or 431A automatic peptide synthesizer (Foster City, CA) employing the chemistry provided by the manufacturer.

Also encompassed by the term NAC are functional fragments or polypeptide analogs thereof. The term "functional fragment" refers to a peptide fragment that is a portion of a full length NAC protein, provided that the portion has one or more biological activities, as defined above, that is characteristic of the corresponding full length NAC. For example, a functional fragment of an invention NAC protein can have one or more of the protein:protein binding activities prevalent in NAC. In addition, the characteristic of a functional fragment of invention NAC proteins to elicit an immune response is useful for obtaining an anti-NAC antibodies. Thus, the invention also provides functional fragments of invention NAC proteins, which can be identified using the binding and routine methods, such as bioassays described herein.

The term "polypeptide analog" includes any polypeptide having an amino acid residue sequence substantially the same as a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the ability to functionally mimic an NAC as described herein. Examples of

conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The amino acid length of functional fragments or polypeptide analogs of the present invention can range from about 5 amino acids up to the full-length protein sequence of an invention NAC. In certain embodiments, the amino acid lengths include, for example, at least about 10 amino acids, at least about 20, at least about 30, at least about 40, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 250 or more amino acids in length up to the full-length NAC protein sequence.

As used herein the phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue, provided that such polypeptide displays the required binding activity. The phrase "chemical derivative" refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized

to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions of residues, relative to the sequence of a polypeptide whose sequence is shown herein, so long as the required activity is maintained.

The present invention also provides compositions containing an acceptable carrier and any of an isolated, purified NAC mature protein or functional polypeptide fragments thereof, alone or in combination with each other. These polypeptides or proteins can be recombinantly derived, chemically synthesized or purified from native sources. As used herein, the term "acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as phosphate buffered saline solution, water and emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The NAC compositions described herein can be used, for example, in methods described hereinafter.

In accordance with another embodiment of the invention, substantially pure nucleic acid molecules, and functional fragments thereof, are provided, which encode

invention NACs. Exemplary invention nucleic acid molecules are those comprising substantially the same nucleotide sequence encoding NACβ (SEQ ID NO: 1), NACγ (SEQ ID NO: 3), and NACδ (SEQ ID NO: 5).

The nucleic acid molecules described herein are useful for producing invention proteins, when such nucleic acids are incorporated into a variety of protein expression systems known to those of skill in the art. In addition, such nucleic acid molecules or fragments thereof can be labeled with a readily detectable substituent and used as hybridization probes for assaying for the presence and/or amount of an invention NAC gene or mRNA transcript in a given sample. The nucleic acid molecules described herein, and fragments thereof, are also useful as primers and/or templates in a PCR reaction for amplifying genes encoding invention proteins described herein.

The term "nucleic acid" (also referred to as polynucleotides) encompasses ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), probes, oligonucleotides, and primers. DNA can be either complementary DNA (cDNA) or genomic DNA, e.g. a gene encoding a NAC. One means of isolating a nucleic acid encoding an NAC polypeptide is to probe a mammalian genomic library with a natural or artificially designed DNA probe using methods well known in the art. DNA probes derived from the NAC gene are particularly useful for this purpose. DNA and cDNA molecules that encode NAC polypeptides can be used to obtain complementary genomic DNA, cDNA or RNA from mammalian (e.g., human, mouse, rat, rabbit, pig, and the like), or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or

genomic libraries, by methods described in more detail below. Such nucleic acids may include, but are not limited to, nucleic acids comprising substantially the same nucleotide sequence as set forth in SEQ ID NOs:1 (NACβ), 3 (NACγ), and 5 (NACδ).

Use of the terms "isolated" and/or "purified" and/or "substantially purified" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been produced in such form by the hand of man, and thus are separated from their native *in vivo* cellular environment, and are substantially free of any other species of nucleic acid or protein. As a result of this human intervention, the recombinant DNAs, RNAs, polypeptides and proteins of the invention are useful in ways described herein that the DNAs, RNAs, polypeptides or proteins as they naturally occur are not.

Invention NAC proteins and nucleic acids encoding such, can be obtained from any species of organism, such as prokaryotes, eukaryotes, plants, fungi, vertebrates, invertebrates, and the like. A particular species can be mammalian, As used herein, "mammalian" refers to a subset of species from which an invention NAC is derived, e.g., human, rat, mouse, rabbit, monkey, baboon, bovine, porcine, ovine, canine, feline, and the like. A preferred NAC herein, is human NAC.

In one embodiment of the present invention, cDNAs encoding the invention NACs disclosed herein comprise substantially the same nucleotide sequence as the coding region set forth in any of SEQ ID NOs :1, 3 and 5. Preferred cDNA molecules encoding the invention proteins

comprise the same nucleotide sequence as the coding region set forth in any of SEQ ID NOs :1, 3 and 5.

As employed herein, the term "substantially the same nucleotide sequence" refers to DNA having sufficient identity to the reference polynucleotide, such that it will hybridize to the reference nucleotide under moderately stringent hybridization conditions. In one embodiment, DNA having substantially the same nucleotide sequence as the reference nucleotide sequence encodes substantially the same amino acid sequence as that set forth in any of SEQ ID NOs:2, 4, 6, 10 or 12. In another embodiment, DNA having "substantially the same nucleotide sequence" as the reference nucleotide sequence has at least 60% identity with respect to the reference nucleotide sequence. DNA having at least 70%, more preferably at least 90%, yet more preferably at least 95%, identity to the reference nucleotide sequence is preferred.

This invention also encompasses nucleic acids which differ from the nucleic acids shown in SEQ ID NOs :1, 3 and 5, but which have the same phenotype. Phenotypically similar nucleic acids are also referred to as "functionally equivalent nucleic acids". As used herein, the phrase "functionally equivalent nucleic acids" encompasses nucleic acids characterized by slight and non-consequential sequence variations that will function in substantially the same manner to produce the same protein product(s) as the nucleic acids disclosed herein. In particular, functionally equivalent nucleic acids encode polypeptides that are the same as those encoded by the nucleic acids disclosed herein or that have conservative amino acid variations. For example, conservative variations include substitution of a

non-polar residue with another non-polar residue, or substitution of a charged residue with a similarly charged residue. These variations include those recognized by skilled artisans as those that do not substantially alter the tertiary structure of the protein.

Further provided are nucleic acids encoding NAC polypeptides that, by virtue of the degeneracy of the genetic code, do not necessarily hybridize to the invention nucleic acids under specified hybridization conditions. Preferred nucleic acids encoding the invention NACs are comprised of nucleotides that encode substantially the same amino acid sequence as set forth in SEQ ID NOs:2, 4, 6, 10 or 12.

Thus, an exemplary nucleic acid encoding an invention NAC may be selected from:

(a) DNA encoding the amino acid sequence set forth in SEQ ID NOs:2, 4, 6, 10 or 12,

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes biologically active NAC, or

(c) DNA degenerate with respect to (b) wherein said DNA encodes biologically active NAC.

Hybridization refers to the binding of complementary strands of nucleic acid (i.e., sense:antisense strands or probe:target-DNA) to each other through hydrogen bonds, similar to the bonds that naturally occur in chromosomal DNA. Stringency levels used to hybridize a given probe with target-DNA can be readily varied by those of skill in the art.

The phrase "stringent hybridization" is used herein to refer to conditions under which polynucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions.

As used herein, the phrase "moderately stringent hybridization" refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60% identity, preferably about 75% identity, more preferably about 85% identity to the target DNA; with greater than about 90% identity to target-DNA being especially preferred. Preferably, moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C.

The phrase "high stringency hybridization" refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65°C (i.e., if a hybrid is not stable in 0.018M NaCl at 65°C, it will not be stable under high stringency conditions, as contemplated herein). High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C.

The phrase "low stringency hybridization" refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 42°C, followed by washing in 1X SSPE, 0.2% SDS, at 50°C. Denhart's solution and SSPE (see, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1989)) are well known to those of skill in the art as are other suitable hybridization buffers.

As used herein, the term "degenerate" refers to codons that differ in at least one nucleotide from a reference nucleic acid, e.g., SEQ ID NOs :1, 3 and 5, but encode the same amino acids as the reference nucleic acid. For example, codons specified by the triplets "UCU", "UCC", "UCA", and "UCG" are degenerate with respect to each other since all four of these codons encode the amino acid serine.

Preferred nucleic acids encoding the invention polypeptide(s) hybridize under moderately stringent, preferably high stringency, conditions to substantially the entire sequence, or substantial portions (i.e., typically at least 15-30 nucleotides) of the nucleic acid sequence set forth in SEQ ID NOs :1, 3 and 5.

The invention nucleic acids can be produced by a variety of methods well-known in the art, e.g., the methods described herein, employing PCR amplification using oligonucleotide primers from various regions of SEQ ID NOs :1, 3 and 5, and the like.

In accordance with a further embodiment of the present invention, optionally labeled NAC-encoding cDNAs, or fragments thereof, can be employed to probe

library(ies) (e.g., cDNA, genomic, and the like) for additional nucleic acid sequences encoding novel NACs. Construction of suitable mammalian cDNA libraries, including mammalian cDNA libraries, is well-known in the art. Screening of such a cDNA library is initially carried out under low-stringency conditions, which comprise a temperature of less than about 42°C, a formamide concentration of less than about 50%, and a moderate to low salt concentration.

Presently preferred probe-based screening conditions comprise a temperature of about 37°C, a formamide concentration of about 20%, and a salt concentration of about 5X standard saline citrate (SSC; 20X SSC contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0). Such conditions will allow the identification of sequences which have a substantial degree of similarity with the probe sequence, without requiring perfect homology. The phrase "substantial similarity" refers to sequences which share at least 50% homology. Preferably, hybridization conditions will be selected which allow the identification of sequences having at least 70% homology with the probe, while discriminating against sequences which have a lower degree of homology with the probe. As a result, nucleic acids having substantially the same nucleotide sequence as SEQ ID NOs :1, 3 and 5 are obtained.

As used herein, a nucleic acid "probe" is single-stranded DNA or RNA, or analogs thereof, that has a sequence of nucleotides that includes at least 15, at least 20, at least 50, at least 100, at least 200, at least 300, at least 400, or at least 500 contiguous bases that are the same as (or the complement of) any contiguous bases set forth in any of SEQ ID NOs :1, 3 and

5. Preferred regions from which to construct probes include 5' and/or 3' coding regions of SEQ ID NOs :1, 3 and 5. In addition, the entire cDNA encoding region of an invention NAC, or the entire sequence corresponding to SEQ ID NOs :1, 3 and 5, may be used as a probe. Probes may be labeled by methods well-known in the art, as described hereinafter, and used in various diagnostic kits.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal. Any label or indicating means can be linked to invention nucleic acid probes, expressed proteins, polypeptide fragments, or antibody molecules. These atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturation to form a fluorochrome (dye) that is a useful immunofluorescent tracer. A description of immunofluorescent analytic techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

In one embodiment, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, and the like. In another embodiment, radioactive elements are employed labeling agents. The linking of a label to a substrate, i.e., labeling of

nucleic acid probes, antibodies, polypeptides, and proteins, is well known in the art. For instance, an invention antibody can be labeled by metabolic incorporation of radiolabeled amino acids provided in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). Conventional means of protein conjugation or coupling by activated functional groups are particularly applicable. See, for example, Aurameas et al., Scand. J. Immunol., Vol. 8, Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Patent No. 4,493,795.

Also provided are antisense-nucleic acids having a sequence capable of binding specifically with full-length or any portion of an mRNA that encodes NAC polypeptides so as to prevent translation of the mRNA. The antisense-nucleic acid may have a sequence capable of binding specifically with any portion of the sequence of the cDNA encoding NAC polypeptides. As used herein, the phrase "binding specifically" encompasses the ability of a nucleic acid sequence to recognize a complementary nucleic acid sequence and to form double-helical segments therewith via the formation of hydrogen bonds between the complementary base pairs. An example of an antisense-nucleic acid is an antisense-nucleic acid comprising chemical analogs of nucleotides.

Compositions comprising an amount of the antisense-nucleic acid, described above, effective to reduce expression of NAC polypeptides by passing through a cell membrane and binding specifically with mRNA encoding NAC polypeptides so as to prevent translation and an acceptable hydrophobic carrier capable of passing through a cell membrane are also provided herein. Suitable hydrophobic carriers are described, for example,

in U.S. Patent Nos. 5,334,761; 4,889,953; 4,897,355, and the like. The acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind to a cell-type specific receptor.

Antisense-nucleic acid compositions are useful to inhibit translation of mRNA encoding invention polypeptides. Synthetic oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding NAC polypeptides and inhibit translation of mRNA and are useful as compositions to inhibit expression of NAC associated genes in a tissue sample or in a subject.

In accordance with another embodiment of the invention, kits are provided for detecting mutations, duplications, deletions, rearrangements and aneuploidies in NAC genes comprising at least one invention probe or antisense nucleotide.

The present invention provides means to modulate levels of expression of NAC polypeptides by employing synthetic antisense-nucleic acid compositions (hereinafter SANC) which inhibit translation of mRNA encoding these polypeptides. Synthetic oligonucleotides, or other antisense-nucleic acid chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to full-length or portions of an NAC coding strand, including nucleotide sequences set forth in SEQ ID NOs :1, 3 and 5 . The SANC is designed to be stable in the blood stream for administration to a subject by injection, or in

laboratory cell culture conditions. The SANC is designed to be capable of passing through the cell membrane in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SANC which render it capable of passing through cell membranes, for example, by designing small, hydrophobic SANC chemical structures, or by virtue of specific transport systems in the cell which recognize and transport the SANC into the cell. In addition, the SANC can be designed for administration only to certain selected cell populations by targeting the SANC to be recognized by specific cellular uptake mechanisms which bind and take up the SANC only within select cell populations. In a particular embodiment the SANC is an antisense oligonucleotide.

For example, the SANC may be designed to bind to a receptor found only in a certain cell type, as discussed *supra*. The SANC is also designed to recognize and selectively bind to target mRNA sequence, which may correspond to a sequence contained within the sequences shown in SEQ ID NOs :1, 3 and 5. The SANC is designed to inactivate target mRNA sequence by either binding thereto and inducing degradation of the mRNA by, for example, RNase I digestion, or inhibiting translation of mRNA target sequence by interfering with the binding of translation-regulating factors or ribosomes, or inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups which either degrade or chemically modify the target mRNA. SANCs have been shown to be capable of such properties when directed against mRNA targets (see Cohen et al., TIPS, 10:435 (1989) and Weintraub, Sci. American, January (1990), pp.40; both incorporated herein by reference).

In accordance with yet another embodiment of the present invention, there is provided a method for the recombinant production of invention NAC or CARD-X by expressing the above-described nucleic acid sequences in suitable host cells. Recombinant DNA expression systems that are suitable to produce NAC described herein are well-known in the art. For example, the above-described nucleotide sequences can be incorporated into vectors for further manipulation. As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof.

Suitable expression vectors are well-known in the art, and include vectors capable of expressing DNA operatively linked to a regulatory sequence, such as a promoter region that is capable of regulating expression of such DNA. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the inserted DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

Prokaryotic transformation vectors are well-known in the art and include pBlueskript and phage Lambda ZAP vectors (Stratagene, La Jolla, CA), and the like. Other suitable vectors and promoters are disclosed in detail in U.S. Patent No. 4,798,885, issued January 17, 1989, the disclosure of which is incorporated herein by reference in its entirety.

Other suitable vectors for transformation of *E. coli* cells include the pET expression vectors (Novagen, see U.S. patent 4,952,496), e.g., pET11a, which contains the T7 promoter, T7 terminator, the inducible *E. coli* lac operator, and the lac repressor gene; and pET 12a-c, which contain the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal. Another suitable vector is the pIN-IIIompA2 (see Duffaud et al., Meth. in Enzymology, 153:492-507, 1987), which contains the lpp promoter, the lacUV5 promoter operator, the ompA secretion signal, and the lac repressor gene.

In accordance with another embodiment of the present invention, there are provided "recombinant cells" containing the nucleic acid molecules (i.e., DNA, cDNA or mRNA) of the present invention. Methods of transforming suitable host cells, preferably bacterial cells, and more preferably *E. coli* cells, as well as methods applicable for culturing said cells containing a gene encoding a heterologous protein, are generally known in the art. See, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual (2 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1989).

Exemplary methods of transformation include, e.g., transformation employing plasmids, viral, or bacterial phage vectors, transfection, electroporation, lipofection, and the like. The heterologous DNA can optionally include sequences which allow for its extrachromosomal maintenance, or said heterologous DNA can be caused to integrate into the genome of the host (as an alternative means to ensure stable maintenance in the host).

Host organisms contemplated for use in the practice of the present invention include those organisms in which recombinant production of heterologous proteins has been carried out. Examples of such host organisms include bacteria (e.g., *E. coli*), yeast (e.g., *Saccharomyces cerevisiae, Candida tropicalis, Hansenula polymorpha* and *P. pastoris;* see, e.g., U.S. Patent Nos. 4,882,279, 4,837,148, 4,929,555 and 4,855,231), mammalian cells (e.g., HEK293, CHO and Ltk$^-$ cells), insect cells, and the like. Presently preferred host organisms are bacteria. The most preferred bacteria is *E. coli*.

In one embodiment, nucleic acids encoding the invention NAC can be delivered into mammalian cells, either *in vivo* or *in vitro* using suitable viral vectors well-known in the art. Suitable retroviral vectors, designed specifically for "gene therapy" methods, are described, for example, in WIPO publications WO 9205266 and WO 9214829, which provide a description of methods for efficiently introducing nucleic acids into human cells. In addition, where it is desirable to limit or reduce the *in vivo* expression of the invention NAC, the introduction of the antisense strand of the invention nucleic acid is contemplated.

For example, in one embodiment of the present invention, adenovirus-transferrin/polylysine-DNA (TfAdpl-DNA) vector complexes (Wagner et al., Proc. Natl. Acad. Sci., USA, 89:6099-6103 (1992); Curiel et al., Hum. Gene Ther., 3:147-154 (1992); Gao et al., Hum. Gene Ther., 4:14-24 (1993)) are employed to transduce mammalian cells with heterologous NAC nucleic acid. Any of the plasmid expression vectors described herein may be employed in a TfAdpl-DNA complex.

In accordance with yet another embodiment of the present invention, there are provided anti-NAC antibodies having specific reactivity with an NAC polypeptides of the present invention. The present invention also provides anti-NACβ, anti-NACγ, anti-NACδ, anti-NACβ-X1, or anti-NACγ/δ-X1 antibodies. It should be recognized that an antibody of the invention can be specific for an epitope that is present only in a particular type of NAC or can be specific for an epitope that is common to more than one type of NAC. For example, an anti-NACδ antibody can be specific for only NACδ or for more than one member of the NAC family. As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain a specific binding activity for a specific antigen of at least about 1 x 105 M-1. One skilled in the art would know that, for example, anti-NACβ antibody fragments or anti-NACγ antibody fragments such as Fab, F(ab')2, Fv and Fd fragments can retain specific binding activity for a NACβ or a NACγ, respectively, and, thus, are included within the definition of an antibody. In addition, the term "antibody" as used herein includes naturally occurring antibodies as well as non-naturally occurring antibodies and fragments of antibodies that retain binding activity. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse et al., Science 246:1275-1281 (1989), which is incorporated herein by reference.

Invention antibodies can be produced by methods known in the art using invention polypeptides, proteins or portions thereof as antigens. For example, polyclonal and monoclonal antibodies can be produced by methods well known in the art, as described, for example, in Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory (1988)), which is incorporated herein by reference. Invention polypeptides can be used as immunogens in generating such antibodies. Alternatively, synthetic peptides can be prepared (using commercially available synthesizers) and used as immunogens. Amino acid sequences can be analyzed by methods well known in the art to determine whether they encode hydrophobic or hydrophilic domains of the corresponding polypeptide. Altered antibodies such as chimeric, humanized, CDR-grafted or bifunctional antibodies can also be produced by methods well known in the art. Such antibodies can also be produced by hybridoma, chemical synthesis or recombinant methods described, for example, in Sambrook et al., supra., and Harlow and Lane, supra. Both anti-peptide and anti-fusion protein antibodies can be used. (see, for example, Bahouth et al., Trends Pharmacol. Sci. 12:338 (1991); Ausubel et al., Current Protocols in Molecular Biology (John Wiley and Sons, NY (1989) which are incorporated herein by reference).

In the case of monoclonal antibodies specific to NAC, it is also contemplated herein that the invention includes hybridomas and any other type of cell line which produces a monoclonal antibody. Methods of preparing hybridomas are described for example, in Sambrook et al., supra., and Harlow and Lane, supra; and preparation of any non-hybridoma cell line producing a monoclonal antibody specific to NAC can be carried out in accordance with the methods known in the art and methods described

herein for protein expression in cells such as bacterial cells, yeast cells, amphibian cells, mammalian cells, and the like.

Antibody so produced can be used, inter alia, in diagnostic methods and systems to detect the level of NAC present in a mammalian, preferably human, body sample, such as tissue or vascular fluid. Such antibodies can also be used for the immunoaffinity or affinity chromatography purification of the invention NAC. In addition, methods are contemplated herein for detecting the presence of an invention NAC protein in a tissue or cell, comprising contacting the cell with an antibody that specifically binds to NAC polypeptides, under conditions permitting binding of the antibody to the NAC polypeptides, detecting the presence of the antibody bound to the NAC polypeptide, and thereby detecting the presence of invention polypeptides. With respect to the detection of such polypeptides, the antibodies can be used for *in vitro* diagnostic or *in vivo* imaging methods.

Immunological procedures useful for *in vitro* detection of target NAC polypeptides in a sample include immunoassays that employ a detectable antibody. Such immunoassays include, for example, ELISA, Pandex microfluorimetric assay, agglutination assays, flow cytometry, serum diagnostic assays and immunohistochemical staining procedures which are well known in the art. An antibody can be made detectable by various means well known in the art. For example, a detectable marker can be directly or indirectly attached to the antibody. Useful markers include, for example, radionucleotides, enzymes, fluorogens, chromogens and chemiluminescent labels.

Invention anti-NAC antibodies are contemplated for use herein to modulate the activity of the NAC polypeptide in living animals, in humans, or in biological tissues or fluids isolated therefrom. The term "modulate" refers to a compound's ability to increase (e.g., via an agonist) or inhibit (e.g., via an antagonist) the biological activity of an invention NAC protein, such as the capablity of binding CARD-containing proteins, NB-ARC-containing proteins, to modulate the activity of proteases such as caspases, to modulate the activity of NF-κB, and to modulate apoptosis. Accordingly, compositions comprising a carrier and an amount of an antibody having specificity for NAC polypeptides effective to inhibit naturally occurring ligands or NAPs from binding to invention NAC polypeptides are contemplated herein. For example, a monoclonal antibody directed to an epitope of an invention NAC polypeptide including an amino acid sequence set forth in SEQ ID NOs:2, 4, 6, 10 or 12, can be useful for this purpose.

The present invention further provides transgenic non-human mammals that are capable of expressing exogenous nucleic acids encoding NAC polypeptides. As employed herein, the phrase "exogenous nucleic acid" refers to nucleic acid sequence which is not native to the host, or which is present in the host in other than its native environment (e.g., as part of a genetically engineered DNA construct). In addition to naturally occurring levels of NAC, invention NAC can either be overexpressed or underexpressed (such as in the well-known knock-out transgenics) in transgenic mammals.

Also provided are transgenic non-human mammals capable of expressing nucleic acids encoding NAC

polypeptides so mutated as to be incapable of normal activity, i.e., do not express native NAC. The present invention also provides transgenic non-human mammals having a genome comprising antisense nucleic acids complementary to nucleic acids encoding NAC polypeptides, placed so as to be transcribed into antisense mRNA complementary to mRNA encoding NAC polypeptides, which hybridizes to the mRNA and, thereby, reduces the translation thereof. The nucleic acid may additionally comprise an inducible promoter and/or tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of nucleic acids are DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID NOs :1, 3 or 5. An example of a non-human transgenic mammal is a transgenic mouse. Examples of tissue specificity-determining elements are the metallothionein promoter and the L7 promoter.

Animal model systems which elucidate the physiological and behavioral roles of NAC polypeptides are also provided, and are produced by creating transgenic animals in which the expression of the NAC polypeptide is altered using a variety of techniques. Examples of such techniques include the insertion of normal or mutant versions of nucleic acids encoding an NAC polypeptide by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos to produce a transgenic animal. (See, for example, Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual (Cold Spring Harbor Laboratory, (1986)).

Also contemplated herein, is the use of homologous recombination of mutant or normal versions of NAC genes

with the native gene locus in transgenic animals, to alter the regulation of expression or the structure of NAC polypeptides (see, Capecchi et al., Science 244:1288 (1989); Zimmer et al., Nature 338:150 (1989); which are incorporated herein by reference). Homologous recombination techniques are well known in the art. Homologous recombination replaces the native (endogenous) gene with a recombinant or mutated gene to produce an animal that cannot express native (endogenous) protein but can express, for example, a mutated protein which results in altered expression of NAC polypeptides.

In contrast to homologous recombination, microinjection adds genes to the host genome, without removing host genes. Microinjection can produce a transgenic animal that is capable of expressing both endogenous and exogenous NAC. Inducible promoters can be linked to the coding region of nucleic acids to provide a means to regulate expression of the transgene. Tissue specific regulatory elements can be linked to the coding region to permit tissue-specific expression of the transgene. Transgenic animal model systems are useful for *in vivo* screening of compounds for identification of specific ligands, i.e., agonists and antagonists, which activate or inhibit NAC protein responses.

A further embodiment of the invention provides a method to identify agents that can effectively alter NAC or CARD-X activity, for example the ability of NAC or CARD-X to associate with one or more heterologous proteins. Thus, the present invention provides a screening assay useful for identifying an effective agent, which can alter the association of a NAC with a NAC associated protein, or a CARD-X with a CARD-X associated protein, such as a CARD-containing protein

and/or an NB-ARC-containing protein. Since invention NAC and CARD-X proteins modulate proteins that are involved in apoptosis (see, e.g. Examples 7.0-13.0), the identification of such effective agents can be useful for modulating the level of apoptosis mediated by NAC-binding or CARD-X-binding proteins in a cell in a subject having a pathology characterized by an increased or decreased level of apoptosis (e.g., cancer, and the like).

Thus, in accordance with the present invention, there are provided methods of: modulating Apaf-1 mediated apoptosis, modulating Apaf-1 induced caspase activation, and modulating Nod-1-induced apoptosis, said methods comprising contacting Apaf-1 with NAC, or a fragment thereof comprising a CARD-domain or NB-domain. Also provided are methods of modulating Bax-mediated apoptosis, of modulating caspase-mediated apoptosis, and of inhibiting Apaf-1 binding to caspase-9, said methods comprising contacting cells with CARD-X or the CARD domain of CARD-X.

Further, since invention NAC and CARD-X proteins comprise CARD domains, effective agents can be useful for modulation of their respective CARD-domain activities (see, e.g. the activities described in Examples 2.0 and 5.0-13.0), in addition to any other CARD-domain activities. These additional CARD domain activities include, for example, NF-κB activity modulation, cytokine receptor signal transduction, and caspase activation/inhibition, regardless of whether the affected caspase is involved in apoptosis or some alternative cellular process such as proteolytic processing and activation of inflammatory cytokines.

As used herein, the term "agent" means a chemical or biological molecule such as a simple or complex organic molecule, a peptide, a peptido-mimetic, a protein or an oligonucleotide that has the potential for altering the association of NAC with a heterologous protein or altering the ability of NAC to self-associate or altering the nucleotide binding and/or hydrolysis activity of NAC. In addition, the term "effective agent" is used herein to mean an agent that can, in fact, alter the association of NAC with a heterologous protein or altering the ability of NAC to self-associate or altering the nucleotide binding and/or hydrolysis activity of NAC. For example, an effective agent may be an anti-NAC antibody or a NAC-associated-protein.

As used herein, the term "alter the association" means that the association between two specifically interacting proteins either is increased or is decreased due to the presence of an effective agent. As a result of an altered association of NAC with another protein in a cell, the activity of the NAC or the NAC associated protein can be increased or decreased, thereby modulating a biological process, for example, the level of apoptosis in the cell. As used herein, the term "alter the activity" means that the agent can increase or decrease the activity of a NAC in a cell, thereby modulating a biological process in a cell, for example, the level of apoptosis in the cell. For example, an effective agent can increase or decrease the NB-ARC:NB-ARC-associating activity of a NAC, without affecting the association of the NAC with a CARD-containing protein. Modulation of the ATP hydrolysis activity can modulate the ability of NAC proteins to associate with other NB-ARC-containing proteins, such as Apaf-1, thereby modulating any process effected by such association between NAC and an

NB-ARC-containing protein. Similarly, the term "alters the association" of NAC with another protein refers to increasing or decreasing, or otherwise changing the association between a NAC and a protein that specifically binds to NAC (i.e., a NAC associated protein).

An effective agent can act by interfering with the ability of a NAC to associate with another protein, or can act by causing the dissociation of NAC from a complex with a NAC-associated protein, wherein the ratio of bound NAC to free NAC is related to the level of a biological process, for example, apoptosis, in a cell. For example, binding of a ligand to a NAC-associated protein can allow the NAC-associated protein, in turn, to bind a NAC. The association, for example, of a CARD-containing protein and a NAC can result in activation or inhibition of the NB-ARC:NB-ARC-associating activity of NAC. In the presence of an effective agent, the association of a NAC and a CARD-containing protein can be altered, which can thereby alter the activation of caspases in the cell. As a result of the altered caspase activation, the level of apoptosis in a cell can be increased or decreased. Thus, the identification of an effective agent that alters the association of NAC with another protein can allow for the use of the effective agent to increase or decrease the level of apoptosis in a cell.

An effective agent can be useful, for example, to increase the level of apoptosis in a cell such as a cancer cell, which is characterized by having a decreased level of apoptosis as compared to its normal cell counterpart. An effective agent also can be useful, for example, to decrease the level of apoptosis in a cell such as a T lymphocyte in a subject having a viral disease such as acquired immunodeficiency syndrome, which

is characterized by an increased level of apoptosis in an infected T cell as compared to a normal T cell. Thus, an effective agent can be useful as a medicament for altering the level of apoptosis in a subject having a pathology characterized by increased or decreased apoptosis. In addition, an effective agent can be used, for example, to decrease the level of apoptosis and, therefore, increase the survival time of a cell such as a hybridoma cell in culture. The use of an effective agent to prolong the survival of a cell *in vitro* can significantly improve bioproduction yields in industrial tissue culture applications.

A NAC that lacks the ability to bind the NB-ARC domain of another protein but retains the ability to self-associate via its CARD domain or to bind to other CARD-containing proteins is an example of an effective agent, since the expression of a non-NB-ARC-associating NAC in a cell can alter the association of a the endogenous NAC protein with itself or with NAC associated proteins.

Thus, it should be recognized that a mutation of a NAC can be an effective agent, depending, for example, on the normal level of NAC/NAC-associated protein that occurs in a particular cell type. In addition, an active fragment of a NAC can be an effective agent, provided the active fragment can alter the association of NAC and another protein in a cell. Such active fragments, which can be peptides as small as about five amino acids, can be identified, for example, by screening a peptide library (see, for example, Ladner et al., U.S. Patent No: 5,223,409, which is incorporated herein by reference) to identify peptides that can bind a NAC-associated protein.

Similarly, a peptide or polypeptide portion of a NAC-associated protein also can be an effective agent. A peptide such as the C-terminal peptide of NAC-associated protein can be useful, for example, for decreasing the association of NAC with a CARD-containing protein or a NB-ARC-containing protein in a cell by competing for binding to the NAC. A non-naturally occurring peptido-mimetic also can be useful as an effective agent. Such a peptido-mimetic can include, for example, a peptoid, which is peptide-like sequence containing N-substituted glycines, or an oligocarbamate. A peptido-mimetic can be particularly useful as an effective agent due, for example, to having an increased stability to enzymatic degradation *in vivo*.

A screening assay to identify an effective agent can be performed *in vivo* using the two hybrid system or can be performed *in vitro* as disclosed herein. The yeast two hybrid system, for example, can be used to screen a panel of agents to identify effective agents that alter the association of NAC or CARD-X with another protein. An effective agent can be identified by detecting an altered level of transcription of a reporter gene. For example, the level of transcription of a reporter gene due to the bridging of a DNA-binding domain and trans-activation domain by a NAP and NAC hybrids can be determined in the absence and in the presence of an agent. An effective agent, which alters the association between NAC or CARD-X and another protein, can be identified by a proportionately altered level of transcription of the reporter gene as compared to the control level of transcription in the absence of the agent.

As understood by those of skill in the art, assay methods for identifying agents that modulate NAC or CARD-

X activity generally require comparison to a control. For example, one type of a "control" is a cell or culture that is treated substantially the same as the test cell or test culture exposed to the agent, with the distinction that the "control" cell or culture is not exposed to the agent. Another type of "control" cell or culture may be a cell or culture that is identical to the transfected cells, with the exception that the "control" cell or culture do not express native proteins. Accordingly, the response of the transfected cell to agent is compared to the response (or lack thereof) of the "control" cell or culture to the same agent under the same reaction conditions. Similarly, a "control" can be the extract, partially purified or not, of a cell not exposed to the agent or not expressing certain native proteins. A "control" may also be an isolated compound, for example, a protein (e.g., Skp-1 as used in Examples), which is known to not specifically associate with NAC proteins.

Accordingly, in accordance with another embodiment of the present invention, there is provided a method of identifying an effective agent that alters the association of a NB-ARC and CARD-containing protein (NAC) with a NAC associated protein (NAP), comprising:

a) contacting said NAC and NAP proteins, under conditions that allow the NAC and NAP proteins to associate, with an agent suspected of being able to alter the association of the NAC and NAP proteins; and

b) detecting the altered association of the NAC and NAP proteins, wherein the altered association identifies an effective agent.

As used herein, the phrase "NAC associated protein" (NAP) refers to proteins that bind directly or indirectly to an invention NAC, such as the NAC interacting and/or binding proteins set forth in Examples 2.0-9.0.

Methods well-known in the art for detecting the altered association of the NAC and NAP proteins, for example, measuring protein:protein binding, protein degradation or apoptotic activity can be employed in bioassays described herein to identify agents as agonists or antagonists of NAC proteins. As described herein, NAC proteins have the ability to self-associate. Thus, methods for identifying effective agents that alter the association of a NAC protein NAP will also be useful for identifying effective agents that alter the ability of NAC to self-associate.

Similarly, CARD-X proteins have the ability to interact with other CARD-containing proteins and to self-associate. Thus, methods for identifying effective agents that alter the association of a NAC and another protein will also be useful for identifying effective agents that alter the ability of CARD-X to self-associate or to associate with a heterologous CARD-containing protein. In accordance with another embodiment of the present invention, there is provided a method of identifying an effective agent that alters the association of a CARD-containing CARD-X protein with a CARD-X associated protein (CAP), comprising:

a) contacting said CARD-X and CAP proteins, under conditions that allow the CARD-X and CAP proteins to associate, with an agent suspected of being able to alter the association of the CARD-X and CAP proteins; and

b) detecting the altered association of the CARD-X and CAP proteins, wherein the altered association identifies an effective agent.

As used herein, the phrase "CARD-X associated protein" (CAP) refers to proteins that bind directly or indirectly to an invention CARD-X, such as the CARD-X interacting and/or binding proteins set forth in Examples 3.0 and 10.0-13.0.

As used herein, "conditions that allow said NAC and NAP proteins to associate" or "conditions that allow the CARD-X and CAP proteins to associate" refers to environmental conditions in which NAC:NAP or CARD-X:CAP specifically associate. Such conditions will typically be aqueous conditions, with a pH between 3.0 and 11.0, and temperature below 100°C. Preferably, the conditions will be aqueous conditions with salt concentrations below the equivalent of 1 M NaCl, and pH between 5.0 and 9.0, and temperatures between 0°C and 50°C. Most preferably, the conditions will range from physiological conditions of normal yeast or mammalian cells, or conditions favorable for carrying out in vitro assays such as immunoprecipitation and GST-NAC:NAP association assays, and the like.

In yet another embodiment of the present invention, there are provided methods for modulating the caspase modulating activity mediated by NAC or CARD-X proteins, the method comprising:

contacting an NAC or CARD-X protein with an effective, modulating amount of an agonist or antagonist identified by the above-described bioassays.

The present invention also provides *in vitro* screening assays. Such screening assays are particularly useful in that they can be automated, which allows for high through-put screening, for example, of randomly or rationally designed agents such as drugs, peptidomimetics or peptides in order to identify those agents that effectively alter the association of NAC and NAP proteins, or CARD-X and CAPs, or the activity of a NAC or CARD-X, and thereby, modulate apoptosis. An *in vitro* screening assay can utilize, for example, a NAC or a NAC fusion protein such as a NAC-glutathione-S-transferase fusion protein (GST/NAC; see Examples). For use in the *in vitro* screening assay, the NAC or NAC fusion protein should have an affinity for a solid substrate as well as the ability to associate with a NAC-associated protein. For example, when a NAC is used in the assay, the solid substrate can contain a covalently attached anti-NAC antibody. Alternatively, a GST/NAC fusion protein can be used in the assay and the solid substrate can contain covalently attached glutathione, which is bound by the GST component of the GST/NAC fusion protein. Similarly, a NAC-associated protein, or a GST/CARD-containing protein or GST/NB-ARC-containing protein fusion protein can be used in an *in vitro* assay as described herein.

Those of skill in the art will recognize that CARD-X and/or CARD-X associated proteins (CAPs) can be utilized analogous to NAC and NAPs in the screening, diagnostic and therapeutic methods described herein.

An *in vitro* screening assay can be performed by allowing a NAC or NAC-fusion protein, for example, to bind to the solid support, then adding a NAC-associated protein and an agent to be tested. Control reactions, which do not contain an agent, can be performed in

parallel. Following incubation under suitable conditions, which include, for example, an appropriate buffer concentration and pH and time and temperature that permit binding of the particular NAC and NAC-associated protein, the amount of protein that has associated in the absence of an agent and in the presence of an agent can be determined. The association of a NAC-associated protein with a NAC protein can be detected, for example, by attaching a detectable moiety such as a radionuclide or a fluorescent label to a NAC-associated protein and measuring the amount of label that is associated with the solid support, wherein the amount of label detected indicates the amount of association of the NAC-associated protein with a NAC protein. An effective agent is determined by comparing the amount of specific binding in the presence of an agent as compared to the control level of binding, wherein an effective agent alters the association of NAC with the NAC-assocated protein. Such an assay is particularly useful for screening a panel of agents such as a peptide library in order to detect an effective agent.

The invention further provides methods for introducing a nucleic acid encoding a NAC into a cell in a subject, for example, for gene therapy. Viruses are specialized infectious agents that can elude host defense mechanisms and can infect and propagate in specific cell types. Viral based systems provide the advantage of being able to introduce relatively high levels of the heterologous nucleic acid into a variety of cells. Suitable viral vectors for introducing invention nucleic acid encoding an NAC protein into mammalian cells (e.g., vascular tissue segments) are well known in the art. These viral vectors include, for example, Herpes simplex virus vectors (e.g., Geller et al., Science,

241:1667-1669 (1988)), Vaccinia virus vectors (e.g., Piccini et al., Meth. in Enzymology, 153:545-563 (1987); Cytomegalovirus vectors (Mocarski et al., in Viral Vectors, Y. Gluzman and S.H. Hughes, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1988, pp. 78-84), Moloney murine leukemia virus vectors (Danos et al., Proc. Natl. Acad. Sci., USA, 85:6469 (1980)), adenovirus vectors (e.g., Logan et al., Proc. Natl. Acad. Sci., USA, 81:3655-3659 (1984); Jones et al., Cell, 17:683-689 (1979); Berkner, Biotechniques, 6:616-626 (1988); Cotten et al., Proc. Natl. Acad. Sci., USA, 89:6094-6098 (1992); Graham et al., Meth. Mol. Biol., 7:109-127 (1991)), adeno-associated virus vectors, retrovirus vectors (see, e.g., U.S. Patent 4,405,712 and 4,650,764), and the like. Especially preferred viral vectors are the adenovirus and retroviral vectors.

Suitable retroviral vectors for use herein are described, for example, in U.S. Patent 5,252,479, and in WIPO publications WO 92/07573, WO 90/06997, WO 89/05345, WO 92/05266 and WO 92/14829, incorporated herein by reference, which provide a description of methods for efficiently introducing nucleic acids into human cells using such retroviral vectors. Other retroviral vectors include, for example, the mouse mammary tumor virus vectors (e.g., Shackleford et al., Proc. Natl. Acad. Sci. USA, 85:9655-9659 (1988)), and the like.

In particular, the specificity of viral vectors for particular cell types can be utilized to target predetermined cell types. Thus, the selection of a viral vector will depend, in part, on the cell type to be targeted. For example, if a neurodegenerative disease is to be treated by increasing the level of a NAC in neuronal cells affected by the disease, then a viral

vector that targets neuronal cells can be used. A vector derived from a herpes simplex virus is an example of a viral vector that targets neuronal cells (Battleman et al., J. Neurosci. 13:941-951 (1993), which is incorporated herein by reference). Similarly, if a disease or pathological condition of the hematopoietic system is to be treated, then a viral vector that is specific for a particular blood cell or its precursor cell can be used. A vector based on a human immunodeficiency virus is an example of such a viral vector (Carroll et al., J. Cell. Biochem. 17E:241 (1993), which is incorporated herein by reference). In addition, a viral vector or other vector can be constructed to express a nucleic acid encoding a NAC or CARD-X in a tissue specific manner by incorporating a tissue-specific promoter or enhancer into the vector (Dai et al., Proc. Natl. Acad. Sci. USA 89:10892-10895 (1992), which is incorporated herein by reference).

For gene therapy, a vector containing a nucleic acid encoding a NAC or CARD-X or an antisense nucleotide sequence can be administered to a subject by various methods. For example, if viral vectors are used, administration can take advantage of the target specificity of the vectors. In such cases, there in no need to administer the vector locally at the diseased site. However, local administration can be a particularly effective method of administering a nucleic acid encoding a NAC or CARD-X. In addition, administration can be via intravenous or subcutaneous injection into the subject. Following injection, the viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection. Injection of viral vectors into the spinal

fluid also can be an effective mode of administration, for example, in treating a neurodegenerative disease.

Receptor-mediated DNA delivery approaches also can be used to deliver a nucleic acid molecule encoding a NAC into cells in a tissue-specific manner using a tissue-specific ligand or an antibody that is non-covalently complexed with the nucleic acid molecule via a bridging molecule (Curiel et al., Hum. Gene Ther. 3:147-154 (1992); Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987), each of which is incorporated herein by reference). Direct injection of a naked or a nucleic acid molecule encapsulated, for example, in cationic liposomes also can be used for stable gene transfer into non-dividing or dividing cells *in vivo* (Ulmer et al., Science 259:1745-1748 (1993), which is incorporated herein by reference). In addition, a nucleic acid molecule encoding a NAC can be transferred into a variety of tissues using the particle bombardment method (Williams et al., Proc. Natl. Acad. Sci. USA 88:2726-2730 (1991), which is incorporated herein by reference). Such nucleic acid molecules can be linked to the appropriate nucleotide sequences required for transcription and translation.

A particularly useful mode of administration of a nucleic acid encoding a NAC or CARD-X is by direct inoculation locally at the site of the disease or pathological condition. Local administration can be advantageous because there is no dilution effect and, therefore, the likelihood that a majority of the targeted cells will be contacted with the nucleic acid molecule is increased. Thus, local inoculation can alleviate the targeting requirement necessary with other forms of administration and, if desired, a vector that infects all

cell types in the inoculated area can be used. If expression is desired in only a specific subset of cells within the inoculated area, then a promotor, an enhancer or other expression element specific for the desired subset of cells can be linked to the nucleic acid molecule. Vectors containing such nucleic acid molecules and regulatory elements can be viral vectors, viral genomes, plasmids, phagemids and the like. Transfection vehicles such as liposomes also can be used to introduce a non-viral vector into recipient cells. Such vehicles are well known in the art.

The present invention also provides therapeutic compositions useful for practicing the therapeutic methods described herein. Therapeutic compositions of the present invention, such as pharmaceutical compositions, contain a physiologically compatible carrier together with an invention NAC or CARD-X (or functional fragment thereof), a NAC or CARD-X modulating agent, such as a compound (agonist or antagonist) identified by the methods described herein, or an anti-NAC or anti-CARD-X antibody, as described herein, dissolved or dispersed therein as an active ingredient. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a mammal or human patient for therapeutic purposes.

As used herein, the terms "pharmaceutically acceptable", "physiologically compatible" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to a mammal without the production of undesirable physiological effects such as nausea, dizziness, gastric upset, and the like.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well known in the art. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however, solid forms suitable for solution, or suspension, in liquid prior to use can also be prepared. The preparation can also be emulsified.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like, as well as combinations of any two or more thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and the like, which enhance the effectiveness of the active ingredient.

The therapeutic composition of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable nontoxic salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid, and the like.

Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium hydroxide, ammonium hydroxide, potassium hydroxide, and the like; and organic bases such as mono-, di-, and tri-alkyl and -aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine, and the like) and optionally substituted ethanolamines (e.g., ethanolamine, diethanolamine, and the like).

Physiologically tolerable carriers are well known in the art. Exemplary liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary additional liquid phases include glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

As described herein, an "effective amount" is a predetermined amount calculated to achieve the desired therapeutic effect, e.g., to modulate the protein degradation activity of an invention NAC or CARD-X protein. The required dosage will vary with the particular treatment and with the duration of desired treatment; however, it is anticipated that dosages between about 10 micrograms and about 1 milligram per kilogram of body weight per day will be used for therapeutic treatment. It may be particularly

advantageous to administer such compounds in depot or long-lasting form as discussed hereinafter. A therapeutically effective amount is typically an amount of an NAC-modulating or CARD-X-modulating agent or compound identified herein that, when administered in a physiologically acceptable composition, is sufficient to achieve a plasma concentration of from about 0.1 μg/ml to about 100 μg/ml, preferably from about 1.0 μg/ml to about 50 μg/ml, more preferably at least about 2 μg/ml and usually 5 to 10 μg/ml. Therapeutic invention anti-NAC antibodies can be administered in proportionately appropriate amounts in accordance with known practices in this art.

Also provided herein are methods of treating pathologies, said method comprising administering an effective amount of an invention therapeutic composition. Such compositions are typically administered in a physiologically compatible composition.

Exemplary diseases related to abnormal cell proliferation contemplated herein for treatment according to the present invention include cancer pathologies, keratinocyte hyperplasia, neoplasia, keloid, benign prostatic hypertrophy, inflammatory hyperplasia, fibrosis, smooth muscle cell proliferation in arteries following balloon angioplasty (restenosis), and the like. Exemplary cancer pathologies contemplated herein for treatment include, gliomas, carcinomas, adenocarcinomas, sarcomas, melanomas, hamartomas, leukemias, lymphomas, and the like.

Methods of treating pathologies of abnormal cell proliferation will include methods of modulating the activity of one or more oncogenic proteins, wherein the

oncogenic proteins specifically interact directly or indirectly with NAC or CARD-X. Methods of modulating the activity of such oncogenic proteins will include contacting the oncogenic protein with a substantially pure NAC, CARD-X, or an active fragment (i.e., oncogenic protein-binding fragment) thereof. This contacting will modulate the activity of the oncogenic protein, thereby providing a method of treating a pathology caused by the oncogenic protein. Further methods of modulating the activity of oncogenic proteins will include contacting the oncogenic protein with an agent, wherein the agent modulates the interactions between NAC and the oncogenic protein.

Also contemplated herein, are therapeutic methods using invention pharmaceutical compositions for the treatment of pathological disorders in which there is too little cell division, such as, for example, bone marrow aplasias, immunodeficiencies due to a decreased number of lymphocytes, and the like. Methods of treating a variety of inflammatory diseases with invention therapeutic compositions are also contemplated herein, such as treatment of sepsis, fibrosis (e.g., scarring), arthritis, graft versus host disease, and the like.

The present invention also provides methods for diagnosing a pathology that is characterized by an increased or decreased level of apoptosis in a cell to determine whether the increased or decreased level of apoptosis is due, for example, to increased or decreased expression of a NAC or CARD-X in the cell or to expression of a variant NAC or CARD-X. The identification of such a pathology, which can be due to altered association of a NAC with a NAC-associated protein, or CARD-X with a CAP, in a cell, can allow for

intervention therapy using an effective agent or a nucleic acid molecule or an antisense nucleotide sequence as described above. In general, a test sample can be obtained from a subject having a pathology characterized by having or suspected of having increased or decreased apoptosis and can be compared to a control sample from a normal subject to determine whether a cell in the test sample has, for example, increased or decreased expression of NAC. The level of a NAC in a cell can be determined by contacting a sample with a reagent such as an anti-NAC antibody or a NAC-associated protein, either of which can specifically bind a NAC. For example, the level of a NAC in a cell can determined by well known immunoassay or immunohistochemical methods using an anti-NAC antibody (see, for example, Reed et al., supra, 1992; see, also, Harlow and Lane, supra, (1988)). As used herein, the term "reagent" means a chemical or biological molecule that can specifically bind to a NAC or to a bound NAC/NAC-associated protein complex. For example, either an anti-NAC antibody or a NAC-associated protein can be a reagent for a NAC, whereas either an anti-NAC antibody or an anti-NAC-associated protein antibody can be a reagent for a NAC/NAC-associated protein complex.

As used herein, the term "test sample" means a cell or tissue specimen that is obtained from a subject and is to be examined for expression of a NAC in a cell in the sample. A test sample can be obtained, for example, during surgery or by needle biopsy and can be examined using the methods described herein to diagnose a pathology characterized by increased or decreased apoptosis. Increased or decreased expression of a NAC in a cell in a test sample can be determined by comparison to an expected normal level for a NAC in a particular

cell type. A normal range of NAC levels in various cell types can be determined by sampling a statistically significant number of normal subjects. In addition, a control sample can be evaluated in parallel with a test sample in order to determine whether a pathology characterized by increased or decreased apoptosis is due to increased or decreased expression of a NAC. The test sample can be examined using, for example, immunohistochemical methods as described above or the sample can be further processed and examined. For example, an extract of a test sample can be prepared and examined to determine whether a NAC that is expressed in a cell in the sample can associate with a NAC-associated protein in the same manner as a NAC from a control cell or whether, instead, a variant NAC is expressed in the cell.

In accordance with another embodiment of the present invention, there are provided diagnostic systems, preferably in kit form, comprising at least one invention nucleic acid encoding NAC, NAC protein, and/or anti-NAC antibody described herein, in a suitable packaging material. In one embodiment, for example, the diagnostic nucleic acids are derived from any of SEQ ID NOs :1, 3 and 5. Invention diagnostic systems are useful for assaying for the presence or absence of nucleic acid encoding NAC in either genomic DNA or in transcribed nucleic acid (such as mRNA or cDNA) encoding NAC.

A suitable diagnostic system includes at least one invention NAC nucleic acid, NAC protein, and/or anti-NAC antibody, preferably two or more invention nucleic acids, proteins and/or antibodies, as a separately packaged chemical reagent(s) in an amount sufficient for at least one assay. Instructions for use of the packaged reagent

are also typically included. Those of skill in the art can readily incorporate invention nucleic probes and/or primers into kit form in combination with appropriate buffers and solutions for the practice of the invention methods as described herein.

As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as invention nucleic acid probes or primers, and the like. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. The packaging material has a label which indicates that the invention nucleic acids can be used for detecting a particular sequence encoding NAC including the nucleotide sequences set forth in SEQ ID NOs :1, 3 and 5 or mutations or deletions therein, thereby diagnosing the presence of, or a predisposition for, cancer. In addition, the packaging material contains instructions indicating how the materials within the kit are employed both to detect a particular sequence and diagnose the presence of, or a predisposition for, cancer.

The packaging materials employed herein in relation to diagnostic systems are those customarily utilized in nucleic acid-based diagnostic systems. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding within fixed limits an isolated nucleic acid, oligonucleotide, or primer of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated nucleic acid, oligonucleotide or primer, or it can be a microtiter plate well to which microgram

quantities of a contemplated nucleic acid probe have been operatively affixed.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

A diagnostic assay should include a simple method for detecting the amount of a NAC in a sample that is bound to the reagent. Detection can be performed by labeling the reagent and detecting the presence of the label using well known methods (see, for example, Harlow and Lane, supra, 1988; chap. 9, for labeling an antibody). A reagent can be labeled with various detectable moieties including a radiolabel, an enzyme, biotin or a fluorochrome. Materials for labeling the reagent can be included in the diagnostic kit or can be purchased separately from a commercial source. Following contact of a labeled reagent with a test sample and, if desired, a control sample, specifically bound reagent can be identified by detecting the particular moiety.

A labeled antibody that can specifically bind the reagent also can be used to identify specific binding of an unlabeled reagent. For example, if the reagent is an anti-NAC antibody, a second antibody can be used to detect specific binding of the anti-NAC antibody. A second antibody generally will be specific for the particular class of the first antibody. For example, if an anti-NAC antibody is of the IgG class, a second antibody will be an anti-IgG antibody. Such second antibodies are readily available from commercial sources.

The second antibody can be labeled using a detectable moiety as described above. When a sample is labeled using a second antibody, the sample is first contacted with a first antibody, then the sample is contacted with the labeled second antibody, which specifically binds to the first antibody and results in a labeled sample.

In accordance with another embodiment of the invention, a method is provided to identify NAC-associated proteins. As used herein, the term "NAC-associated protein" or "NAP" means a protein that can specifically bind directly or indirectly, preferably bind directly, to NAC or its alternative isoforms. Because NAC proteins are known to self-associate, NAC proteins are encompassed by the term NAP. An exemplary NAP is a protein or a polypeptide portion of a protein that can bind the NB-ARC, CARD, LRR, or TIM-Barrel-like domains of NAC. Similarly, the term "CARD-X Associated Protein" or "CAP" refers to a protein that can specifically bind directly or indirectly, preferably bind directly, to the CARD-X protein. Likewise, since CARD-X proteins are known to self-associate, CARD-X proteins are encompassed by the term CAP. A NAP or CAP can be identified, for example, using *in vitro* protein binding assays similar to those described in the Examples, by Yeast Two-Hybrid assays similar to those described in the Examples, or by other types of protein-interaction assays and methods.

Using NAC or CARD-X, it is clear to one skilled in the art of protein purification, protein interaction cloning, or protein mass-spectrometry, that NAPs or CAPs can be identified using the methods disclosed herein.

Although the term "NAP" or "CAP" is used generally, it should be recognized that a NAP or CAP that is identified using an assay described herein can be a portion of a protein, which is considered to be a candidate NAP or CAP. As used herein, the term "active fragment" of a NAP or CAP refers to a protein that corresponds to a polypeptide sequence that can bind NAC or CARD-X, respectively, but that consists of only a portion of the full length protein. Although such polypeptides are considered NAPs or CAPs, it is well known that a cDNA sequence obtained from a cDNA library may not encode the full length protein. Thus, a cDNA can encode a polypeptide that is only a portion of a full length protein but, nevertheless, assumes an appropriate conformation and contains a sufficient region so as to bind NAC or CARD-X. However, in the full length protein, the polypeptide can assume a conformation that does not bind NAC or CARD-X, due for example to steric blocking of the NAP or CAP binding site. Such a full length protein is also an example of a NAP or CAP, wherein NAC-binding or CARD-X-binding activity can be activated under the appropriate conditions (i.e., phosphorylation, proteolysis, protein binding, pH change, and the like). For convenience of discussion, the terms "NAP" and "CAP", as used herein, are intended to include a NAP or CAP, respectively, and active fragments thereof.

Since CARD-containing proteins are commonly involved in apoptosis, the association of a NAP or CAP with NAC or CARD-X can affect the level of apoptosis in a cell. The identification by use of the methods described herein of various NAPs or CAPs can provide the necessary insight into cell death or signal transduction pathways controlled by NAC or CARD-X, allowing for the development of assays that are useful for identifying agents that

effectively alter the association of a NAP with NAC or a CAP with CARD-X. Such agents can be useful, for example, for providing effective therapy for a cancer in a subject or for treating an autoimmune disease. These same assays can be used for identification of agents that modulate the self-association of NAC via its CARD domain, NB-ARC domain, or other domains within this protein; and, they can be used for identification of agents that modulate the self-association of CARD-X with itself via its CARD domain or other domains found within this protein.

In a normal cell, a steady state level of association of NAP and NAC proteins likely occurs. This steady state level of association of NAP and NAC proteins in a particular cell type can determine the normal level of apoptosis in that cell type. An increase or decrease in the steady state level of association of NAP and NAC proteins in a cell can result in an increased or decreased level of apoptosis in the cell, which can result in a pathology in a subject. The normal association of NAP and NAC proteins in a cell can be altered due, for example, to the expression in the cell of a variant NAP or NAC protein, respectively, either of which can compete with the normal binding function of NAC and, therefore, can decrease the association of NAP and NAC proteins in a cell. The term "variant" is used generally herein to mean a protein that is different from the NAP or NAC protein that normally is found in a particular cell type. In addition, the normal association of NAP and NAC proteins in a cell can be increased or decreased due, for example, to contact of the cell with an agent such as a drug that can effectively alter the association of NAP and NAC proteins in a cell.

NB-ARC and CARD domain proteins of the invention, NACβ, NACγ and NACδ, were characterized, for example, using an *in vitro* binding assay and CARD-containing proteins were further characterized using the yeast two hybrid system. An *in vivo* transcription activation assay such as the yeast two hybrid system is particularly useful for identifying and manipulating the association of proteins. In addition, the results observed in such an assay likely mirror the events that naturally occur in a cell. Thus, the results obtained in such an *in vivo* assay can be predictive of results that can occur in a cell in a subject such as a human subject.

A transcription activation assay such as the yeast two hybrid system is based on the modular nature of transcription factors, which consist of functionally separable DNA-binding and trans-activation domains. When expressed as separate proteins, these two domains fail to mediate gene transcription. However, transcription activation activity can be restored if the DNA-binding domain and the trans-activation domain are bridged together due, for example, to the association of two proteins. The DNA-binding domain and trans-activation domain can be bridged, for example, by expressing the DNA-binding domain and trans-activation domain as fusion proteins (hybrids), provided that the proteins that are fused to the domains can associate with each other. The non-covalent bridging of the two hybrids brings the DNA-binding and trans-activation domains together and creates a transcriptionally competent complex. The association of the proteins is determined by observing transcriptional activation of a reporter gene (see Example I).

The yeast two hybrid systems exemplified herein use various strains of S. cerevisiae as host cells for vectors that express the hybrid proteins. A transcription activation assay also can be performed using, for example, mammalian cells. However, the yeast two hybrid system is particularly useful due to the ease of working with yeast and the speed with which the assay can be performed. For example, yeast host cells containing a lacZ reporter gene linked to a LexA operator sequence were used to demonstrate that the $CARD_L$ domain of NAC (amino acid residues 1128-1473 of SEQ ID NO:2) can interact with several CARD-containing proteins (see Examples). For example, in one case the DNA-binding domain consisted of the LexA DNA-binding domain, which binds the LexA promoter, fused to the $CARD_L$ domain of NAC and the trans-activation domain consisted of the B42 acidic region separately fused to several cDNA sequences which encoded CARD-containing proteins. When the LexA domain was non-covalently bridged to a trans-activation domain fused to a CARD-containing protein, the association activated transcription of the reporter gene.

A NAP, for example, a CARD-containing protein or an NB-ARC-containing protein also can be identified using an *in vitro* assay such as an assay utilizing, for example, a glutathione-S-transferase (GST) fusion protein as described in the Examples. Such an *in vitro* assay provides a simple, rapid and inexpensive method for identifying and isolating a NAP. Such an *in vitro* assay is particularly useful in confirming results obtained *in vivo* and can be used to characterize specific binding domains of a NAP. For example, a GST/$CARD_L$ fusion protein can be expressed and can be purified by binding to an affinity matrix containing immobilized glutathione. If desired, a sample that can contains a CARD-containing

protein or active fragments of a CARD-containing protein can be passed over an affinity column containing bound GST/$CARD_L$ and a CARD-containing protein that binds to $CARD_L$ can be obtained. In addition, GST/$CARD_L$ can be used to screen a cDNA expression library, wherein binding of the GST/$CARD_L$ fusion protein to a clone indicates that the clone contains a cDNA encoding a CARD-containing protein.

In another embodiment of the invention, methods are provided for monitoring the progress of treatment for a pathology that is characterized by an increased or decreased level of apoptosis in a cell, which methods are useful to ascertain the feasability of such treatment. Monitoring such a therapy, such as, e.g., a therapy that alters association of a NAC with a NAC-associated protein in a cell using an effective agent, can allow for modifications in the therapy to be made, including decreasing the amount of effective agent used in therapy, increasing the amount of effective agent, or using a different effective agent. In general, a test sample can be obtained from a subject having a pathology characterized by increased or decreased apoptosis, which sample can be compared to a control sample from a normal subject to determine whether a cell in the test sample has, for example, increased or decreased expression of NAC. Preferably, this control sample is a previous sample from the same patient, thereby providing a direct comparison of changes to the pathology as a result of the therapy. The level of a NAC in a cell can be determined by contacting a sample with a reagent such as an anti-NAC antibody or a NAC-associated protein, either of which can specifically bind a NAC. For example, the level of a NAC in a cell can determined by well known immunoassay or immunohistochemical methods using an anti-NAC antibody

(see, for example, Reed et al., supra, 1992; see, also, Harlow and Lane, supra, (1988)).

In accordance with another embodiment of the invention, there are provided methods for determining a prognosis of disease free or overall survival in a patient suffering from cancer. For example, it is contemplated herein that abnormal levels of NAC proteins (either higher or lower) in primary tumor tissue show a high correlation with either increased or decreased tumor recurrence or spread, and therefore indicates the likelihood of disease free or overall survival. Thus, the present invention advantageously provides a significant advancement in cancer management because early identification of patients at risk for tumor recurrence or spread will permit aggressive early treatment with significantly enhanced potential for survival. Also provided are methods for predicting the risk of tumor recurrence or spread in an individual having a cancer tumor; methods for screening a cancer patient to determine the risk of tumor metastasis; and methods for determining the proper course of treatment for a patient suffering from cancer. These methods are carried out by collecting a sample from a patient and comparing the level of NAC expression in the patient to the level of expression in a control or to a reference level of NAC expression as defined by patient population sampling, tissue culture analysis, or any other method known for determining reference levels for determination of disease prognosis. The level of NAC expression in the patient is then classified as higher than the reference level or lower than the reference level, wherein the prognosis of survival or tumor recurrence is different for patients with higher levels than the prognosis for patients with lower levels.

All U.S. patents and all publications mentioned herein are incorporated in their entirety by reference thereto. The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLES

1.0 *Cloning cDNA encoding invention NAC proteins.*

Jurkat total RNA was reverse-transcribed to complementary DNAs using MMLV reverse transcriptase (Stratagene) and random hexanucleotide primers. Three overlapping cDNA fragments of NAC were amplified from the Jurkat complementary DNAs with Turbo *Pfu* DNA polymerase (Stratagene) using the following oligonucleotide primer sets: primer set 1; 5'-CCGAATTCACCATGGCTGGCGGAGCCTGGGGC-3' (forward; SEQ ID NO:13) and 5'-CCGCTCGAGTCAACAGAGGGTTGTGGTGGTCTTG-3' (reverse; SEQ ID NO:14), primer set 2; 5'-CCCGAATTCGAACCTCGCATAGTCATACTGC-3' (forward; SEQ ID NO:15) and 5'-GTCCCACAACAGAATTCAATCTCAACGGTC-3' (reverse; SEQ ID NO:16), and primer set 3; 5'-TGTGATGAGAGAAGCGGTGAC-3' (forward; SEQ ID NO:17) and 5'-CCGCTCGAGCAAAGAAGGGTCAGCCAAAGC-3' (reverse; SEQ ID NO:18). The resultant cDNA fragments were ligated into mammalian expression vector pcDNA-myc (Invitrogen, modified as described in Roy et al., EMBO J. 16:6914-6925 (1997)) and assembled to full-length cDNA by ligating fragments 2 and 3 at the EcoRI site to make fragment 4, and by ligating fragments 1 and 4 at the Bst X1 site. Sequencing analysis of the assembled full-length cDNA was carried out and is set forth in SEQ ID NO:2.

Additional NAC cDNAs were obtained which represent alternative mRNA splicing products that encode shorter proteins lacking a 31 amino acid segment (SEQ ID NO:5 and 6), a 45 amino acid segment, or lacking the 31 and 45 amino acid segments (SEQ ID NOs:3 and 4), both located between the LRR and CARD (shown as hatched boxes Figure 1A, and as italiczed amino acids 957-987 and 1261-1305, respectively, in Figure 1B and SEQ ID NO:2). A schematic diagram of the full-length NAC proteins, including three alternatively spliced isoforms lacking regions underlined (amino acids 957-987 or 1261-1305 of SEQ ID NO:2, or lacking both, respectively), are presented in Figure 2. The full length nucleotide sequences of three of these isoforms is set forth in SEQ ID NOs:1, 3, and 5 corresponding to NACβ (full length), NACγ (lacking both splice regions) and NACδ (lacking 31 amino acid splice region), respectively.

Comparison of NAC to known protein sequences using Clustal multiple sequence alignment (Thompson et al., Nucleic Acids Research 22:4673-4680 (1994)) revealed that the CARD domain of NAC near the C-terminus (see, e.g., residues 1373 to 1473 of SEQ ID NO:2) is similar to numerous CARD domain proteins. Thus, unlike the CED-4 family proteins heretofore identified, the CARD domain of NAC is located at its carboxyl- rather than amino-terminus. Further sequence analysis predicted an $\alpha_8\beta_8$ (TIM)-Barrel-like domain similar to those observed in aldolase and RuBisCo in NAC, located on the immediate amino terminal side of the predicted CARD domain (see, e.g., residues 1079 to 1364 of SEQ ID NO:2). Additionally, a portion of NAC was found to have sequence portions homologous to NB-ARC domains (see, e.g., residues 329 to 547 of SEQ ID NO:2) and a leucine-rich

repeat region (see, e.g., residues 808 to 947 of SEQ ID NO:2). Based on its homology to the above proteins the protein of the invention has been termed a NAC protein, as it is a NB-ARC and CARD domain containing protein. ClustalW multiple sequence alignment with other NB-ARC and CARD domain containing proteins confirmed the homology of NAC to other proteins in both the NB-ARC region (particularly in the P-loop, or Walker A, and Walker B portions) and CARD region (Figure 1C and Figure 1D, respectively). This sequence analysis represents the first time a domain resembling a TIM-barrel domain has been identified in a protein that also contains a CARD domain, and also the first time a domain resembling a TIM-barrel domain has been identified in a protein that also contains an NB-ARC domain.

The NB-ARC domain (also referred to as NB-domain) of NAC contains classical Walker A and B boxes indicative of ATP-binding proteins, and is most similar in amino acid sequence to the NB-domain of Nod1 (29%)(Inohara et al. (1999) *J. Biol. Chem.* 274, 14560-7; and Bertin et al. (1999) *J. Biol. Chem.* 274(19), 12955-12958), followed by human Apaf-1 (17%), the *Drosophila* Apaf-1 homologue (12%), and the *C. elegans* CED-4 protein (12%) (Figure 1C). The CARD domain of NAC shares 21%, 19%, and 8% amino acid identity with the CARD domains of Nod1, human Apaf-1, and CED-4, respectively (Figure 1D). The NAC CARD sequence was readily threaded onto the structures of other CARDs, including those reported previously for Apaf-1, pro-caspase-9, and Raidd (Figure 1E), suggesting conservation of the 6 α-helical fold typical of these domains.

NAC mRNAs were widely expressed in adult human tissues, with highest levels found in peripheral blood

leukocytes, thymus, spleen and heart. Two or more closely migrating mRNA species were observed in Northern blots, with the prevalent mRNA species having a length of ≈5 kb.

2.0 *In vitro Protein Binding Assays*.

CARD domains of invention NAC proteins represent homotypic protein interaction domains mediating associations with themselves or other CARDs, wherein specificity for protein-interaction partners is dictated by complementarity in the patterns of hydrophilic and hydrophobic amino-acid residues displayed on the surfaces of these domains. To explore which CARDs the CARD domain of NAC is capable of binding, in vitro protein binding assays were performed. For these experiments, the CARD of NAC was produced in bacteria as a GST-fusion protein, purified, and immobilized on glutathione-Sepharose for testing interactions with various radiolabeled CARD-family proteins, which were produced by in vitro translation.

Complementary DNA encoding the CARD domain of NAC was amplified from Jurkat cDNAs with Turbo *Pfu* DNA polymerase (Stratagene) and primer set 3 as described above. The resultant PCR fragments were digested with *EcoRI* and *Xho I* restriction enzymes and ligated into pGEX-4T1 (Pharmacia) and pcDNA-myc vectors. This region of NAC contains two alternatively spliced isoforms, termed $CARD_L$ (amino acid residues 1128-1473 of SEQ ID NO:2) and $CARD_S$ (amino acid residues 1128-1261 and 1306-1473 of SEQ ID NO:2). The region of cDNA encoding NB-ARC domain was PCR-amplified using primers SEQ ID NO:15 (forward) and SEQ ID NO:14 (reverse). The resultant PCR fragment was digested with EcoRI and Xho I

restriction enzymes (yielding a fragment encoding amino acid residues 326-551 of SEQ ID NO:2) and ligated into a pGEX-4T1 and pcDNA-myc vectors.

NB-ARC, $CARD_L$, and $CARD_S$ in pGEX-4T1 were expressed in XL-1 blue *E. coli* cells (Stratagene), and affinity-purified using glutathione (GSH)-sepharose according to known methods, such as those in Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley and Sons (1999). For GST pull-down assays, purified $CARD_L$ and $CARD_S$ GST fusion proteins and GST alone (0.1-0.5 µg immobilized on 10-15 µl GSH-sepharose beads) were incubated with 1 mg/ml of BSA in 100 µl Co-IP buffer [142.4 mM KCl, 5mM M_gCl_2, 10 mM HEPES (pH 7.4), 0.5 mM EGTA, 0.2% NP-40, 1 mM DTT, and 1 mM PMSF] for 30 min. at room temperature. The beads were then incubated with 1 µl of rat reticulocyte lysates (TnT-lysate; Promega, Inc.) containing ^{35}S-labeled, *in vitro* translated $CARD_L$, $CARD_S$, or control protein Skp-1 in 100 µl Co-IP buffer supplemented with 0.5 mg/ml BSA for overnight at 4°C. The beads were washed four times in 500 µl Co-IP buffer, followed by boiling in 20 µl Laemmli-SDS sample buffer. The eluted proteins were analyzed by SDS-PAGE. The bands of SDS-PAGE gels were detected by fluorography.

The resultant homodimerization pattern reveals that $CARD_L$-$CARD_L$, $CARD_S$-$CARD_S$, and both $CARD_L$-$CARD_S$ containing lanes have very strong signals, whereas lanes containing control GST alone and control Skp-1 have negligible signals. Thus, CARD domains of the invention NAC show a very strong ability to self-associate *in vitro*.

In addition, the CARD of NAC displayed specific interactions in GST pull-down assays with CARD-containing regions of Apaf-1, CED-4, and itself, but not with a

variety of other CARD-containing proteins, including pro-caspases-1, 2, 9, 11, Raidd (Cradd), Cardiak (RIP2; Rick), cIAP1, cIAP2, or Bcl-10 (CIPER; hE10) (Figure 4A). Similar results were obtained by yeast two-hybrid methods as set forth below. Thus, the CARD domain of NAC selectively binds members of the CED-4 family, but does not interact significantly with CARD-carrying caspases or a variety of CARD-containing adapter proteins.

3.0 *Protein Interaction Studies in Yeast.*

EGY48 yeast cells (*Saccharomyces cerevisiae*: MATα, trp1, ura3, his, leu2::plexApo6-leu2) were transformed with pGilda-CARDL plasmids (his marker) encoding the LexA DNA binding domain fused to: CARD domains of NAC ($CARD_L$) and caspase-9; Apaf-1 without its WD domain; Bcl-XL, Bax and Bcl-2 without transmembrane domains. EGY48 were also transformed with vector pJG4-5 (trp1 marker) encoding the above listed group of proteins and additionally vRas and FADD as target proteins, fused to B42 transactivation domain, and the cells were transformed with a LexA-LacZ reporter plasmid pSH1840 (ura3 marker,), as previously described (Durfee et al., 1993; Sato et al., 1995). Sources for cells and plasmids were described previously in U.S. Patent 5,632,994, and in Zervous et al., Cell 72:223-232 (1993); Gyuris et al., Cell 75:791-803 (1993); Golemis et al., In Current Protocols in Molecular Biology (ed. Ausubel et al.; Green Publ.; NY 1994), each of which is incorporated herein by reference. Transformants were replica-plated on Burkholder's minimal medium (BMM) plates supplemented with leucine and 2% glucose as previously described (Sato et al., Gene 140:291-292 (1994)). Protein-protein interactions were scored by growth of transformants on leucine deficient BMM plates containing 2% galactose and 1% raffinose.

Protein-protein interactions were also evaluated using β-galactosidase activity assays. Colonies grown on BMM/Leu/Glucose plates were filter-lifted onto nitrocellulose membranes, and incubated over-night on BMM/Leu/galactose plates. Yeast cells were lysed by soaking filters in liquid nitrogen and thawing at room temperature. β-galactosidase activity was measured by incubating the filter in 3.2 ml Z buffer (60 mM, Na_2HPO_4, 40 mM Na_2HPO_4, 10 mM KCl, 1 mM $MgSO_4$) supplemented with 50 µl X-gal solution (20mg/ml). Levels of β-galactosidase activity were scaled according to the intensity of blue color generated for each transformant.

As set forth above, the CARD of NAC selectively binds members of the CED-4 family, such as Apaf-1, but does not interact significantly with CARD-carrying caspases or a variety of CARD-containing adapter proteins.

Similar two-hybrid interaction experiments have been performed using the CARD domain of the CARD-X protein. Table I summarizes the results of the two-hybrid experiments wherein a fusion protein containing the DNA-binding domain of the LexA protein expressed from the pGilda plasmid and a CARD domain from CARD-X or several other CARD-containing proteins, including CARDIAK, NAC ($CARD_L$), Apaf-1, caspases-2, 9, and 11, were expressed in the sames cells as CARD domains from CARD-X, CARDIAK, NAC($CARD_L$), caspase-9 and cIAP-2, expressed as fusion proteins with a transactivation domain from the B42 protein from the pJG4-5 plasmid, as described above. As shown, the CARD domain of CARD-X interacted with itself but not with the CARD domains of other proteins.

TABLE I

Yeast Two Hybrid Analysis of CARD-X:CARD interactions

	pGilda	pJG4-5	Results
1	CARD-X CARD	CARD-X-CARD	+++
2	CARD-X CARD	CARDIAK	-
3	CARD-X CARD	NAC-$CARD_L$	-
4	CARD-X CARD	Caspase-9 CARD	-
5	CARD-X CARD	cIAP-2	-
6	CARDIAK	CARD-X CARD	-
7	NAC-$CARD_L$	CARD-X CARD	-
8	APAF C3+C4	CARD-X CARD	-
9	Caspase-2	CARD-X CARD	-
10	Caspase-11	CARD-X CARD	-
11	Caspase 9-C-terminus	CARD-X CARD	-
12	CARDIAK	CARDIAK	++++

4.0 ***Self-Association of NB-ARC domain of NAC.***

To explore the characteristics of the NB-domain of NAC, in vitro binding experiments were performed using a glutathione-S-transferase (GST) fusion protein containing the putative NB-domain of NAC. *In vitro* translated, ^{35}S-labeled rat reticulocyte lysates (1 µl) containing NB-ARC or pro-Caspase-9 (used as a control) were incubated with GSH-sepharose beads conjugated with purified GST-NB-ARC or GST alone for a GST pull-down assay, resolved on SDS-PAGE and visualized by fluorography as described above. One tenth of input were

loaded for NB-ARC or pro-Caspase-9 as controls. The NB-domain of NAC self-associated in experiments where binding of GST-NB to ^{35}S-labeled in vitro translated (IVT) NAC NB-domain was assayed (Figure 3A). Thus, in this assay, the NB-ARC-containing fragment of NAC demonstrates a strong ability to homodimerize (Figure 3A).

In contrast, the NB-domain of NAC failed to bind a variety of other proteins, including pro-caspase-9, Apaf-1, and Ced-4 (Figure 3A). Pretreatment of samples with apyrase to deplete ATP or addition of non-hydrolyzable γ-S-ATP prevented self-association of the NB-domain of NAC (Figure 3B, 3C), consistent with ATP-dependent oligomerization as reported previously for Apaf-1 and CED-4. Mutating a lysine residue in the Walker A box motif (P-loop motif), which is known to be critical for nucleotide triphosphate binding by other NB-domains (Chinnaiyan et al. (1997) *Nature* 388, 728-729), greatly diminished the ability of the NB-domain of NAC to self-associate (Figure 3D). The results, therefore, indicate that the NB-domain of NAC functions analogous to CED-4 family proteins as an ATP-dependent self-association domain.

The ability to self-associate and to bind other known CARD domains establishes the CARD domains of NAC, $CARD_S$ and $CARD_L$, as capable of the same protein-protein interactions observed in other known CARD domains. The ability of CARD-X to self-associate also establishes this protein as having the same protein-protein interaction properties of known CARD proteins. Thus two isoforms of a new human CARD domain have been characterized, and a highly related sequence of another human protein CARD-X has also been characterized. In addition, the ability of the putative NB-ARC domain of NAC has been shown to both

self-associate, establishing this domain as capable of the same protein-protein interactions observed in other known NB-ARC domains. Therefore, the NAC protein has been demonstrated to contain both a functional CARD domain and a functional NB-ARC domain.

5.0 Protein-Protein Interactions of NAC.

The ability of NAC to interact with itself, Apaf-1, Nod1, and CED-4 in cells was confirmed by co-immunoprecipitation (Figure 4B, C). For these experiments, NAC containing a myc-epitope tag was co-expressed by transient transfection in HEK293T cells with HA-tagged NAC, Flag-Apaf-1, HA-Apaf-1 lacking the WD domains (ΔWD), HA-CED-4, Flag-Nod1 or various control proteins (Figure 4B, 4C). The myc-NAC protein was then recovered by immunoprecipitation and the resulting immune-complexes were analyzed by SDS-PAGE/immunoblotting for the presence of associated HA- or Flag-tagged proteins.

The results show that NAC, Apaf-1, Nod1, and CED-4 all associated with NAC in these co-immunoprecipitation experiments, whereas pro-caspase-9, cIAP1, cIAP2, Bcl-10, and Akt did not (see, e.g., Figures 4B and C). Interestingly, compared to full-length Apaf-1, NAC co-immunoprecipitated more efficiently with a truncation mutant of Apaf-1 which lacks the WD-repeat domains that normally maintain this protein in an auto-repressed state, suggesting that "activated" Apaf-1 interacts preferentially with NAC.

6.0 Gel-Sieve Chromatography Analysis of NAC:Apaf-1 binding.

The association of NAC with Apaf-1 was also confirmed by gel-sieve chromatography, using cytosolic extracts which had been stimulated with Cyt-c and dATP to induce Apaf-1 activation and apoptosome assembly. For estimating the size of NAC/Apaf-1 protein complexes, 293T cells were transiently co-transfected with Flag-epitope tagged Apaf-1 and myc-tagged NAC. Cytosolic extracts were prepared from the transfected cells using hypotonic, detergent-free buffer as described in (Deveraux et al. (1997) *Nature* 388, 300-304) and incubated (1.5 mg) with cyt-c (10 μM) and dATP (1 mM) for 5 min at 30°C. The treated protein lysates were immediately fractionated by using a Superose-6 HR 10/30 gel-filtration column in elution buffer containing 50 mM Tris, (pH 7.4), 100 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, and 1 mM DTT. Column fractions (0.5 ml) were analyzed by SDS-PAGE, followed by immunoblotting using anti-Flag M2 antibody (for Apaf-1) and anti-myc antibody (for NAC).

Analysis of fractions from the molecular-sieve column revealed that nearly all the Apaf-1 and NAC co-migrated together in a huge complex, with an estimated molecular mass in excess of 2 MDa (Figure 4D). Using these column fractions for co-immunoprecipitation analysis demonstrated that NAC and Apaf-1 are indeed associated in a common protein-complex, and do not merely co-migrate in an unassociated fashion. Though some NAC and Apaf-1 could be co-immunoprecipitated together in unstimulated extracts, addition of Cyt-c and dATP increased the association of these proteins by $\geq$ 5 fold (Figure 4E). Moreover, binding of NAC to Cyt-c-activated Apaf-1 was rapid, becoming maximal within 1 minute, and transient, returning to baseline levels or less in ~ 30 minutes. Addition of a broad-spectrum caspase inhibitor, 100 μM benozyl-Valinyl-Alaninyl-Aspartyl-

fluoromethylketone (zVAD-fmk), prolonged the stability of the NAC/Apaf-1 complex, suggesting the existence of a post-caspase activation feedback mechanism.

7.0 NAC regulates Apaf-1 apoptotic activity.

Cytosolic extracts containing endogenous Apaf-1 provide a convenient system of studying mechanisms of Cyt-c-induced caspase activation. The effects of NAC on Apaf-1-mediated caspase activation were therefore interrogated using cell extracts which were prepared under isotonic, detergent-free conditions to avoid rupture of mitochondria and release of endogenous Cyt-c (Deveraux et al., supra).

Cytosolic extracts were prepared from 293T cells as described in (Deveraux et al., supra) and incubated (10 µg) with various concentrations of cyt-c and 1 mM dATP in Caspase buffer for 30 min at 30°C. In some cases, extracts were absorbed with GST-fusion proteins immobilized on glutathione-Sepharose overnight at 4°C prior to addition of cyt-c or 10 ng Granzyme-B (Calbiochem). Caspase substrate Ac-DEVD-AFC (100 µM) (CalBiochem) was then added, and protease activity was measured continuously by monitoring the release of fluorigenic AFC at 37°C. Alternatively, transfected cells were directly lysed in Caspase Lysis buffer (10 mM HEPES, pH7.4, 25 mM NaCl, 0.25% Triton X-100, and 1 mM EDTA), normalized for protein content, and monitored for cleavage of Ac-DEVD-AFC as described. Processing of IVT [^{35}S] pro-Caspase-9 in cytosolic extracts was monitored by SDS-PAGE as described in (Cardone et al. (1998) *Science* 282, 1318-1321).

Cytosolic extracts were prepared from HEK293 cells which had been transfected with plasmids producing NAC protein (Figure 5A) or antisense NAC transcripts (Figure 5B). The antisense RNA expression plasmid for NAC was constructed by inserting a cDNA fragment corresponding to amino acids 1-1127 of NAC (SEQ ID NO:2) in pcDNA3-myc in reverse orientation at the *EcoRI* site.

Addition to extracts of exogenous Cyt-c resulted in proteolytic processing of pro-caspase-9 (the direct target of Apaf-1) and activation of downstream caspases which cleave the tetrapeptide substrate Asp-Glu-Val-Asp (DEVD). Over-expression of NAC enhanced Cyt-c-induced processing of pro-caspase-9 and activation of DEVD-cleaving caspases (Figure 5A). In contrast, antisense-mediated reduction in NAC resulted in reduced caspase activation by Cyt-c (Figure 5B). Further evidence that NAC regulates the Apaf-1-dependent activation of caspases was obtained by pre-adsorption of extracts with recombinant purified fragments of NAC (Figure 5C), resulting in suppression of Cyt-c-induced activation of caspases and thus suggesting that the CARD and NB-domains of NAC affinity-deplete proteins of relevance to the Apaf-1/cyt-c apoptosome from extracts. In contrast, the activation of caspases by the serine-protease Granzyme B (GraB) was only slightly affected by pre-adsorption of extracts with NAC fragments (Figure 5D), demonstrating the specificity of these results.

8.0 NAC enhances Apaf-1-induced caspase activation and apoptosis in intact cells.

Having observed in Example 8.0 above that NAC can modulate the function of Apaf-1 in cell-extracts, whether NAC and Apaf-1 can collaborate in inducing caspase activation and apoptosis in intact cells, using transient transfection assays was assayed. 293T cells were transfected with pEGFP (0.1 μg) and plasmids encoding pro-Caspase-9 (0.05 μg), Apaf-1 (0.05 or 2.0 μg) or NAC (0.5, 1, or 2 μg). Total DNA input was normalized with empty vector. After culturing 1.5 days in media containing reduced serum (0.1% fetal bovine serum), floating and adherent cells (recovered by trypsinization) were pooled, fixed in 3.7% formaldehyde/PBS, stained with 1 μg/ml 4',6-diamidino-2-phenylindole (DAPI) and the percentage of GFP-positive cells with apoptotic morphology (nuclear fragmentation, chromatin condensation) was determined by fluorescence microscopy (mean ± SE, n=3) as described in Deveraux et al., supra and Cardone et al., supra.

For these experiments, conditions were devised where transfection of suboptimal amounts of plasmids encoding pro-caspase-9 and Apaf-1 into HEK293T (Figure 6) or HT1080 cells resulted in only a slight increase in apoptosis. A plasmid encoding NAC was then co-transfected in various amounts into these cells. Over-expression of NAC by itself or in combination with pro-caspase-9 had little effect on apoptosis (Figure 6A). In contrast, NAC produced dose-dependent, synergistic induction of apoptosis in combination with Apaf-1 (Figure 6A). Synergistic caspase activation by NAC and Apaf-1 was also demonstrated (Figure 6B). Moreover, the NAC-induced enhancement in Apaf-1 function in cells was correlated with NAC-induced increases in the association of Apaf-1 with pro-caspase-9, based on co-immunoprecipitation experiments showing that more Apaf-1

can be recovered in anti-caspase-9 immunoprecipitates when NAC is over-expressed in the cells (Figure 6C).

While full-length NAC enhanced apoptosis and caspase activation induced by over-expressing the combination of Apaf-1 and pro-caspase-9, fragments of NAC containing only the CARD or NB-domain had the opposite effect, interfering with apoptosis induced by the combination of Apaf-1 and pro-caspase-9 transfection (Figure 6D). Apoptosis induced by a mitochondria/Cyt-c-dependent cell death stimulus, staurosporine, was also suppressed by the dominant-inhibitory CARD and NB fragments of NAC, whereas apoptosis triggered by an Apaf-1-independent stimulus, Fas, was not affected (Figure 6D). Thus, these results indicate that NAC specifically modulates apoptosis pathways governed by Apaf-1.

9.0 NAC enhances Nod1-induced apoptosis.

Since NAC associates with the CED-4 family member, Nod1 (Figure 4C), the effects of co-expressing NAC full-length protein or a fragment representing only the CARD of NAC on apoptosis induced by over-expression of Nod1 was assayed. For experiments with full-length NAC, conditions were identified in which transfection of suboptimal amounts of Nod1 in combination with pro-caspase-9 induced only a slight increase in apoptosis in either HEK293 T cells (Figure 7A) or HT1080 cells. Various amounts of plasmid DNA encoding full-length NAC were then co-transfected. By itself or in combination with pro-caspase-9, full-length NAC had little effect on apoptosis. In combination with sub-optimal amounts of Nod1, however, NAC induced a dose-dependent increase in apoptosis (Figure 7A).

In contrast to full-length NAC which enhanced Nod1-induced apoptosis, a fragment of NAC containing only the CARD domain corresponding to amino acids 1129-1473 of SEQ ID NO:2 suppressed Nod1-indcuced apoptosis. For testing the effects of the CARD domain of NAC, conditions were identified in which higher amounts of Nod1 plasmid were employed, inducing > 75% apoptosis in combination with pro-caspase-9. Various amounts of plasmid DNA encoding the CARD of NAC were then co-transfected, demonstrating a dose-dependent decrease in Nod1-induced apoptosis (Figure 7A). Immunoblotting experiments demonstrated that neither full-length NAC (Figure 7B) nor the CARD domain fragment altered the levels of Nod1 or pro-caspase-9, suggesting that NAC modulates the activity rather than the levels of these proteins.

In addition, co-immunoprecipitation experiments provided indirect evidence that NAC, Nod1, and pro-caspase-9 form a multiprotein complex. As shown in Figure 7B, Nod1 was readily co-immunoprecipitated with procaspase-9 (lane 4), consistent with reports that these proteins are capable of associating. In contrast, very little NAC was present in anti-caspase-9 immune-complexes prepared from cell-lysates in which Nod1 was not over-expressed (lane 5), consistent with our results indicating that NAC does not directly bind pro-caspase-9. However, when Nod1 and NAC were co-expressed in cells, immunoprecipitation of procapase-9 revealed association of both Nod1 and NAC with the immune-complexes (lane 6), suggesting that Nod1 bridges NAC to pro-caspase-9. These results indicated that NAC is capable of associating with and modulating the proapoptotic function of Nod1.

10.0 CARD-X Interacts with caspase-9 via its CARD domain.

Full length CARD-X was constructed using PCR from an EST clone (KIA0955) into the EcoR1/Xhol sites of a pCDNA3 vector containing a Myc tag at its N-terminal end. The same vector was used to clone in CARD-X lacking its CARD domain, and clone in the CARD domain alone corresponding to amino acids 345-431 of SEQ ID NO:8.

HEK293T cells were transfected either with pCDNA3 Myc-tagged full-length CARD-X, CARD-X ΔCARD or with the CARD domain alone in the presence or absence of pCDNA3 Flag-tagged caspase9 C/A mutant. After 48 hrs, cells were lysed in HKMEN (10mM Hepes buffer, 142.5 mM KCl, 5 mM MgCl2, 1 mM EGTA and 0.2 % NP-40) buffer and subjected to immunprecipitation. Anti-myc tagged protein sepharose beads. Samples were incubated at 4^0 C for 1 hr, washed 2x in HKMEN and boiled for 5 min in SDS-sample brffer. Samples were then separated on a 10% SDS-PAGE gel and transferred onto a PVDF membrane which was blocked in 5%milk/BSA for 1 hr followed by incubation with anti-caspase 9 or anti-CARD-X anti-peptide antibodies. Membranes were developed using ECL detection system.

The results indicate that full-length CARD-X and the CARD domain alone interact with Caspase-9 via the CARD domain.

11.0 Effect of CARD-X on Bax-mediated apoptosis.

Full-length CARD-X, CARD-X ΔCARD, and the CARD-X CARD domain were expressed as Myc-tagged proteins by transient transfection in HEK293 cells in the presence (5 to 8) or absence (1 to 4) of pcDNA3 Bax and compared with Bax alone (lane 5). Cells were harvested after 24 hrs, fixed with 3.7% paraformaldehye and stained with DAPI. The percentage of apoptosis was quantitated after

microscopic observation. The results are shown in Figure 8 and indicate that full-length CARD-X and the CARD domain of CARD-X inhibit Bax-mediated apoptosis by approximately 70%.

12.0 Effect of CARD-X on caspase9-mediated apoptosis.

HEK293 cells were transiently transfected with wild type flag-tagged caspase 9 and Apaf-1 in the presence or absence of full-length CARD-X, CARD-X ΔCARD, and the CARD-X CARD proteins. Cells were harvested after 24 hrs, fixed with 3.7% paraformaldehyde and stained with DAPI. The percentage apoptosis was quantitated after microscopic observation. The results are shown in Figure 9 and indicate that full-length CARD-X and the CARD domain of CARD-X inhibit Caspase-9-mediated apoptosis by approximately 50%.

13.0 CARD-X Competes with Apaf-1 for binding Caspase-9.

Flag-tagged caspase9 and Myc-tagged Apaf-1 (1-560') were transiently cotransfected into 293T cells for 48 hrs. Cells were lysed in 0.2% HKMEN and subjected to immunoprecipitation with anti-myc sepharose beads. Beads were washed in HKMEN follwed by incubation in the absence (lane 1) or presence (lanes 2 to 5) of increasing amounts of lysates from 293T cells previously transfected with full-length CARD-X. Samples were washed and proteins separated on SDS-polyacrylamide gels, blotted to PVDF membranes and immunoblotted with anti-caspase9 antibodies. The results indicate that CARD-X can competively inhibit the binding of Apaf-1 to Caspase-9.

Although the invention has been described with

reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

That which is claimed is:

1. Isolated nucleic acid encoding a NB-ARC and CARD containing protein (NAC), or functional fragments thereof, selected from:

(a) DNA encoding the amino acid sequence set forth in SEQ ID NOs:2, 4 or 6, or

(b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes biologically active NAC, or

(c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes biologically active NAC.

2. A nucleic acid according to claim 1, wherein said nucleic acid hybridizes under high stringency conditions to the NAC coding portion of any of SEQ ID NOs:1, 3 and 5.

3. A nucleic acid according to claim 1, wherein the nucleotide sequence of said nucleic acid is substantially the same as set forth in any of SEQ ID NO:1, 3 and 5.

4. A nucleic acid according to claim 1, wherein the nucleotide sequence of said nucleic acid is the same as that set forth in any of SEQ ID NOs:1, 3 and 5.

5. A nucleic acid according to claim 1, wherein said nucleic acid is cDNA.

6. A vector containing the nucleic acid of claim 1.

7. Recombinant cells comprising the nucleic acid of claim 1.

8. An oligonucleotide comprising at least 15 nucleotides capable of specifically hybridizing with a the nucleotide sequence set forth in any of SEQ ID NOs:1, 3 and 5.

9. An oligonucleotide according to claim 8, wherein said oligonucleotide is labeled with a detectable marker.

10. An antisense-nucleic acid capable of specifically binding to mRNA encoded by said nucleic acid according to claim 1.

11. A kit for detecting the presence of the NAC cDNA sequence comprising at least one oligonucleotide according to claim 9.

12. An isolated NAC protein comprising a NB-ARC domain, a CARD domain and a TIM-Barrel-like domain.

13. The protein of claim 12, further comprising a LRR domain.

14. An isolated protein according to claim 12, wherein the amino acid sequence of said protein comprises substantially the same sequence as any of SEQ ID NOs:2, 4 or 6.

15. A NAC according to claim 14 comprising the same amino acid sequence as set forth in any of SEQ ID NOs:2, 4 or 6.

16. A NAC according to claim 14, wherein said protein is encoded by a nucleotide sequence comprising substantially the same nucleotide sequence as set forth in SEQ ID NOs:1, 3 or 5.

17. A NAC according to claim 14, wherein said protein is encoded by a nucleotide sequence comprising the same sequence as set forth in SEQ ID NOs:1, 3 or 5.

18. A method for expression of a NAC protein, said method comprising culturing cells of claim 7 under conditions suitable for expression of said NAC.

19. An isolated anti-NAC antibody having specific reactivity with a NAC according to claim 12.

20. Antibody according to claim 19, wherein said antibody is a monoclonal antibody.

21. A cell line producing the monoclonal antibody of claim 20.

22. An antibody according to claim 19, wherein said antibody is a polyclonal antibody.

23. A composition comprising an amount of the antisense-nucleic acid according to claim 10 effective to inhibit expression of a human NAC and an acceptable hydrophobic carrier capable of passing through a cell membrane.

24. A transgenic nonhuman mammal expressing exogenous nucleic acid according to claim 1, encoding a NAC.

25. A transgenic nonhuman mammal according to claim 24, wherein said nucleic acid encoding said NAC has been mutated, and wherein the NAC so expressed is not native NAC.

26. A transgenic nonhuman mammal according to claim 24, wherein the transgenic nonhuman mammal is a mouse.

27. A method for identifying nucleic acids encoding a mammalian NAC or CARD-X, said method comprising:

contacting a sample containing nucleic acids with an oligonucleotide according to claim 8 or SEQ ID NO:7, wherein said contacting is effected under high stringency hybridization conditions, and identifying compounds which hybridize thereto.

28. A method for detecting the presence of a human NAC in a sample, said method comprising contacting a test sample with an antibody according to claim 19, detecting the presence of an antibody:NAC complex, and therefor detecting the presence of a human NAC in said test sample.

29. Single strand DNA primers for amplification of NAC nucleic acid, wherein said primers comprise a nucleic acid sequence derived from the nucleic acid sequences set forth as SEQ ID NOs:1, 3 and 5.

30. A method for modulating the activity of an oncogenic protein, comprising contacting said oncogenic proteins with a substantially pure NAC or CARD-X, or an oncogenic protein-binding fragment thereof.

31. A method of identifying an effective agent that alters the association of a NAC with a NAC associated protein (NAP), or a CARD-X with a CARD-X asociated protein (CAP), comprising the steps of:

a) contacting said NAC and NAP proteins, or CARD-X and CAP proteins, under conditions that allow said NAC and NAP, or CARD-X and CAP, proteins to associate with an agent suspected of being able to alter the association of said NAC and NAP, or CARD-X and CAP, proteins; and

b) detecting the altered association of said NAC and NAP, or CARD-X and CAP, proteins, wherein said altered association identifies an effective agent.

32. The method of claim 31, wherein said altered association is detected by measuring the transcriptional activity of a reporter gene.

33. The method of claim 31, wherein said NAC has nucleotide binding activity.

34. The method of claim 31, wherein said effective agent is a drug.

35. The method of claim 31, wherein said effective agent is a protein.

36. A method for modulating an activity mediated by a NAC or CARD-X protein, said method comprising:

contacting said NAC or CARD-X protein with an effective, modulating amount of an agent identified by claim 31.

37. The method of claim 36, wherein said modulated activity is selected from the group consisting of: binding of NAC or CARD-X to a CARD-containing protein; binding of NAC to a NB-ARC-containing protein; binding of NAC to a LRR-containing protein; and caspase proteolytic activity.

38. A method of modulating the level apoptosis in a cell, comprising the steps of:

a) introducing a nucleic acid molecule encoding a NAC or CARD-X into the cell; and

b) expressing said NAC or CARD-X in said cell, wherein the expression of said NAC or CARD-X modulates apoptosis in said cell.

39. A method of modulating the level of apoptosis in a cell, comprising introducing an antisense nucleotide sequence into the cell, wherein said antisense nucleotide sequence specifically hybridizes to a nucleic acid molecule encoding a NAC or CARD-X, wherein said hybridization reduces or inhibits the expression of said NAC or CARD-X in said cell.

40. A therapeutic composition comprising a compound selected from a NAC, a CARD-X, or functional fragment thereof, an agent identified according to claim 31, or an

anti-NAC antibody; and a pharmaceutically acceptable carrier.

41. A method of treating a pathology characterized by abnormal cell proliferation or abnormal inflammation, said method comprising administering an effective amount of the composition according to claim 40.

42. A method of diagnosing a pathology characterized by an increased or decreased level of a NAC or CARD-X in a subject, comprising the steps of:

a) obtaining a test sample from the subject;

b) contacting said test sample with an agent that can bind said NAC or CARD-X under suitable conditions, which allow specific binding of said agent to said NAC or CARD-X; and

c) comparing the amount of said specific binding in said test sample with the amount of specific binding in a control sample, wherein an increased or decreased amount of said specific binding in said test sample as compared to said control sample is diagnostic of a pathology.

43. The method of claim 42, wherein said agent is an anti-NAC antibody, a NAC-associated-protein (NAP), or a CARD-X associated protein.

44. A method of modulating the level of apoptosis in a cell, comprising contacting the cell with an agent that effectively alters the association of NAC with a NAC-associated-protein or of CARD-X with a CARD-X

associated protein, in the cell, or that effectively alters the activity of a NAC or CARD-X in the cell.

45. A chimeric protein comprising a domain selected from the group consisting of the NB-ARC domain of the NAC of claim 14 and the CARD of the NAC of claim 14.

46. An isolated protein comprising a TIM-Barrel-like domain and a second domain selected from the group consisting of a CARD domain, a NB-ARC domain, and a LRR domain.

47. The chimeric protein of claim 45, comprising the NB-ARC domain of SEQ ID NO:2 and the CARD domain of SEQ ID NO:8.

48. The method of claim 31, wherein said agent modulates CARD:CARD association or NB-ARC:NB-ARC association.

49. A method of modulating CARD:CARD interactions comprising contacting a NAC protein with the agent of claim 48.

50. The method of claim 31, wherein said agent modulates transcription.

51. The method of claim 50, wherein said agent modulates NF-κB activity.

52. A method of modulating transcription comprising contacting a cell with a compound selected from the group consisting of: a NAC protein or functional fragment thereof, an agent identified according to claim 31, and an anti-NAC antibody.

53. A method of diagnosing cancer or monitoring cancer therapy comprising contacting a test sample from a patient with the antibody of claim 19.

54. A method of assessing prognosis of patients with cancer comprising contacting a test sample from a patient with the antibody of claim 19.

55. An effective agent that binds a nucleotide binding site of NAC.

56. An effective agent that modulates the association of NAC or CARD-X with a pro-caspase or a caspase.

57. The method of claim 56, wherein said pro-caspase is pro-caspase-8 and said caspase is caspase-8.

58. The method of claim 56, wherein said pro-caspase is pro-caspase-9 and said caspase is caspase-9.

59. The method of claim 56, wherein said effective agent inhibits the association of said NAC or CARD-X with said pro-caspase or said caspase.

60. The method of claim 56, wherein said effective agent increases the association of said NAC or CARD-X with said pro-caspase or said caspase.

61. An effective agent that modulates the association of NAC or CARD-X with a CED-4 family protein.

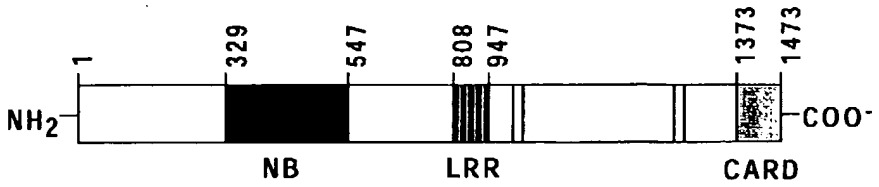
62. The method of claim 61, wherein said CED-4 family protein is selected from the group consisting of CED-4, Apaf-1, Dark, and CARD4/nod1.

63. The method of claim 61, wherein said CED-4 family protein is Apaf-1.

64. The method of claim 61, wherein said effective agent inhibits the association of said NAC with said CED-4 family protein.

65. The method of claim 61, wherein said effective agent increases the association of said NAC with said CED-4 family protein.

A



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121 IHELPAGCTQGSERRVLRQLPDTSGRRWREISASLLYQALPSSPDHESPSQESPNAPTST
181 AVLGSWGSPPQPSLAPREQEAPGTQWPLDETSGIYYTEIRERERERKSEKGRPPWAAVVGT
241 PPQAHTSLQPHHHPWEPSVRESLCSTWPWKNEDFNQKFTQLLLLQRPHPRSQDPLVKRSW
                                    P-loop (Walker A)
301 PDYVEENRGHLIEIRDLFGPGLDTQEPR[IVILQGAAGIGKS]TLARQVKEAWGRGQLYGDR
                                                  Walker B
361 FQHVFYFSCRELAQSKVVSLAELIGKDGTATPAPIRQILSRPERL[IFILDGVDE]PGWVLQ
421 EPSSELCLHWSQPQPADALLGSLLGKTILPEASFLITARTTALQNLIPSLEQARWVEVLG
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601 ISTFLKMGILQEHPIPLSYSFIHLCFQEFFAAMSYVLEDEKGRGKHSNCIIDLEKTLEAY
661 GIHGLFGASTTRFLLGLLSDEGEREMENIFHCRLSQGRNLMQWVPSLQLLLQPHSLESLH
721 CLYETRNKTFLTQVMAHFEEMGMCVETDMELLVCTFCIKFSRHVKKLQLIEGRQHRSTWS
781 PTMVVLFRWVPVTDAYWQILFSVLKVTRNLKELDLSGNSLSHSAVKSLCKTLRRPRCLLE
841 TLRLAGCGLTAEDCKDLAFGLRANQTLTELDLSFNVLTDAGAKHLCQRLRQPSCKLQRLQ
901 LVSCGLTSDCCQDLASVLSASPSLKELDLQQNNLDDVGVRLLCEGLRHPACKLIRLGLDQ
961 TTLSDEMRQELRALEQEKPQLLIFSRRKPSVMTPTEGLDTGEMSNSTSSLKRQRLGSERA
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FIGURE 1

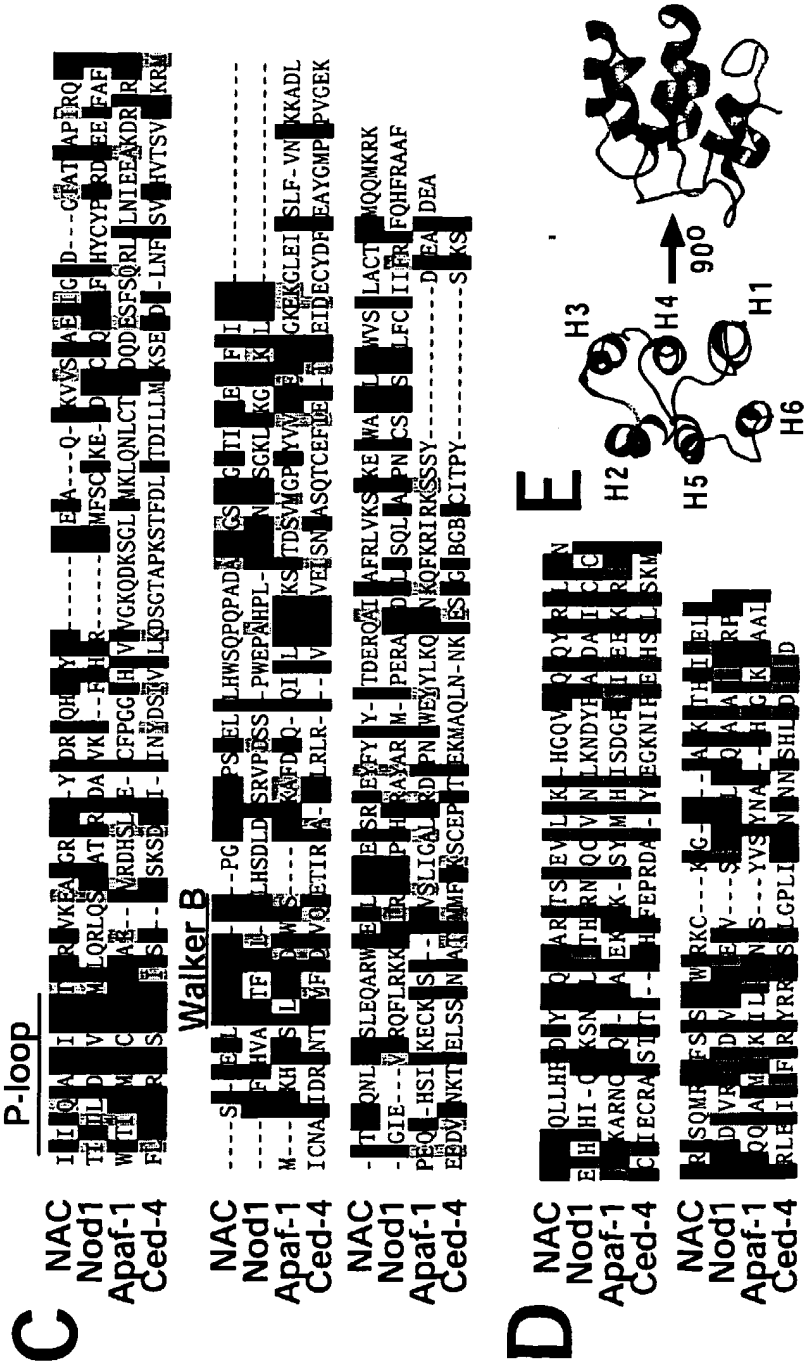
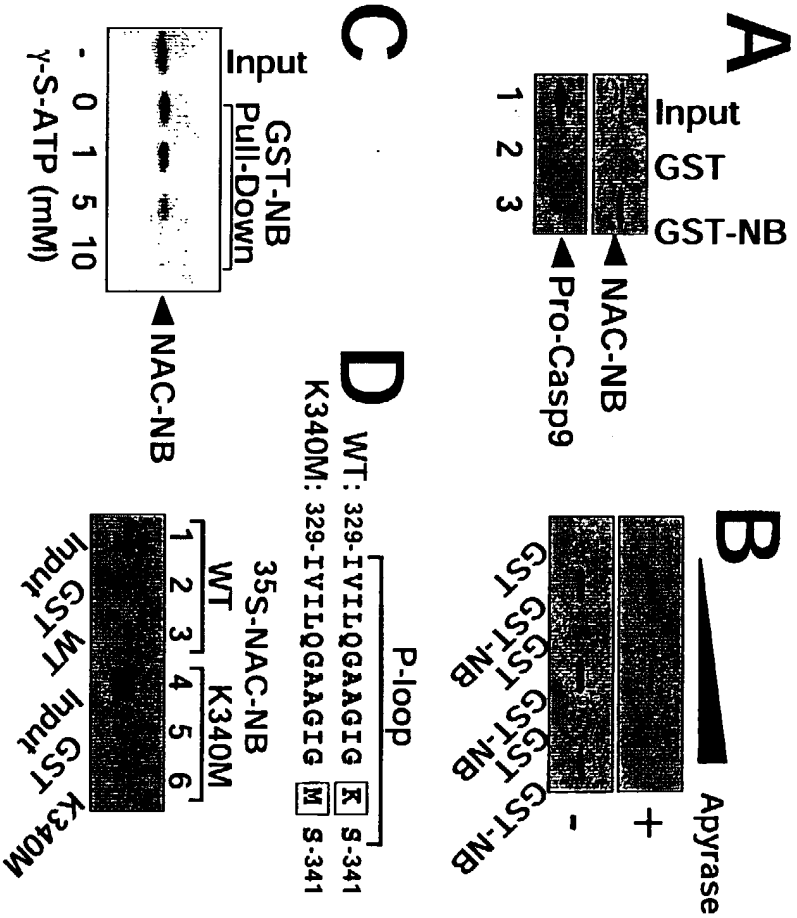


FIGURE 1 CONTINUED

FIGURE 2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Input
GST
GST-NB
1 2 3
Pro-Casp9
NAC-NB
B
Apyrase
+
-
GST
GST-NB
GST
GST-NB
GST
GST-NB
C
Input
GST-NB
Pull-Down
NAC-NB
- 0 1 5 10
γ-S-ATP (mM)
D
WT: 329-IVILQGAAGIG K S-341
K340M: 329-IVILQGAAGIG M S-341
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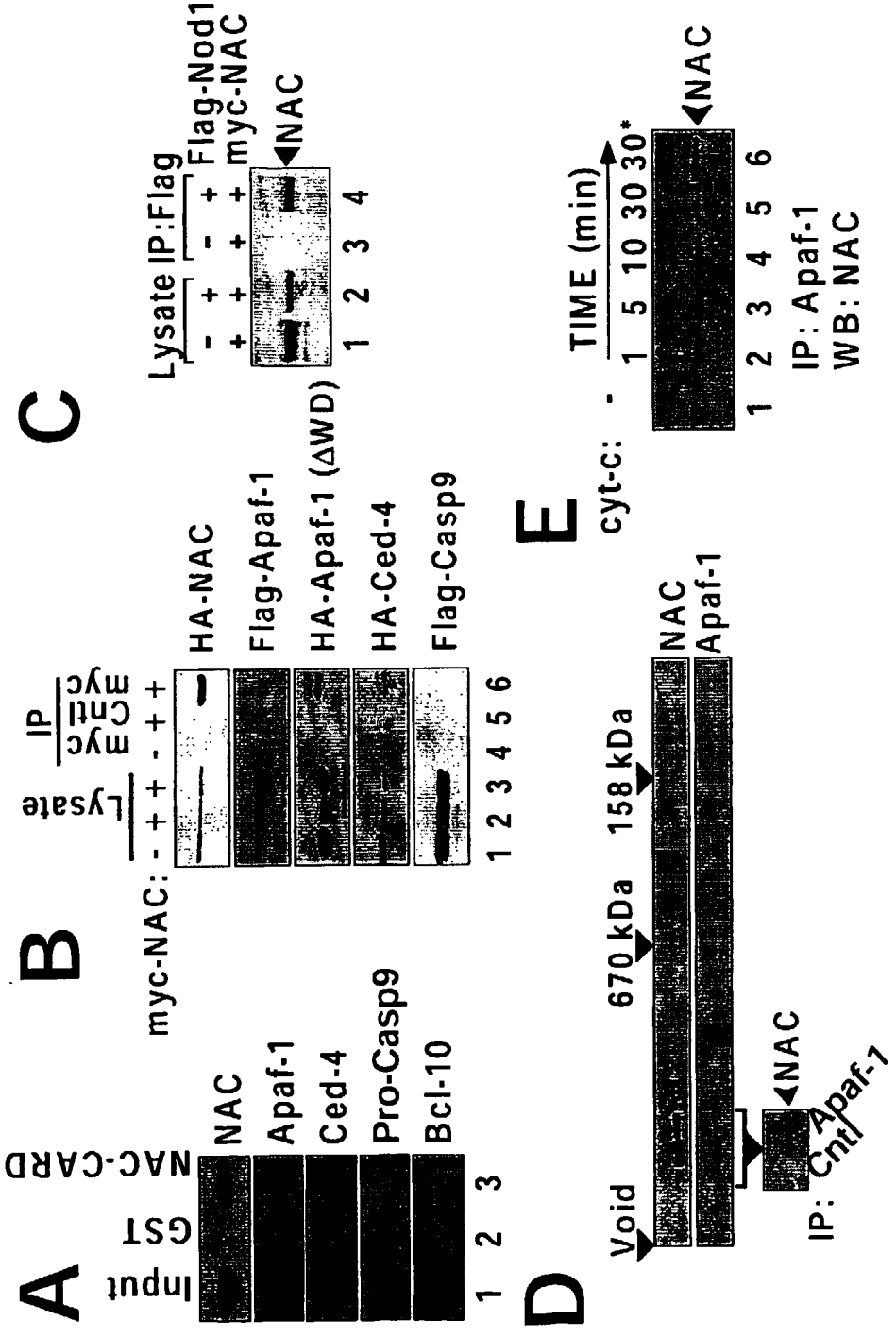
K340M
GST
Input
WT
GST
Input

FIGURE 3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Input
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NAC
Apaf-1
Ced-4
Pro-Casp9
Bcl-10
1 2 3
B
myc-NAC: - + + - + +
Lysate
IP
myc
Cntl
myc
HA-NAC
Flag-Apaf-1
HA-Apaf-1 (ΔWD)
HA-Ced-4
Flag-Casp9
1 2 3 4 5 6
C
Lysate IP:Flag
- + + - + +
+ + + + + +
Flag-Nod1
myc-NAC
NAC
1 2 3 4
D
Void
670 kDa
158 kDa
NAC
Apaf-1
NAC
IP: Cntl Apaf-1
E
cyt-c: -
TIME (min)
1 5 10 30 30*
NAC
1 2 3 4 5 6
IP: Apaf-1
WB: NAC

FIGURE 4

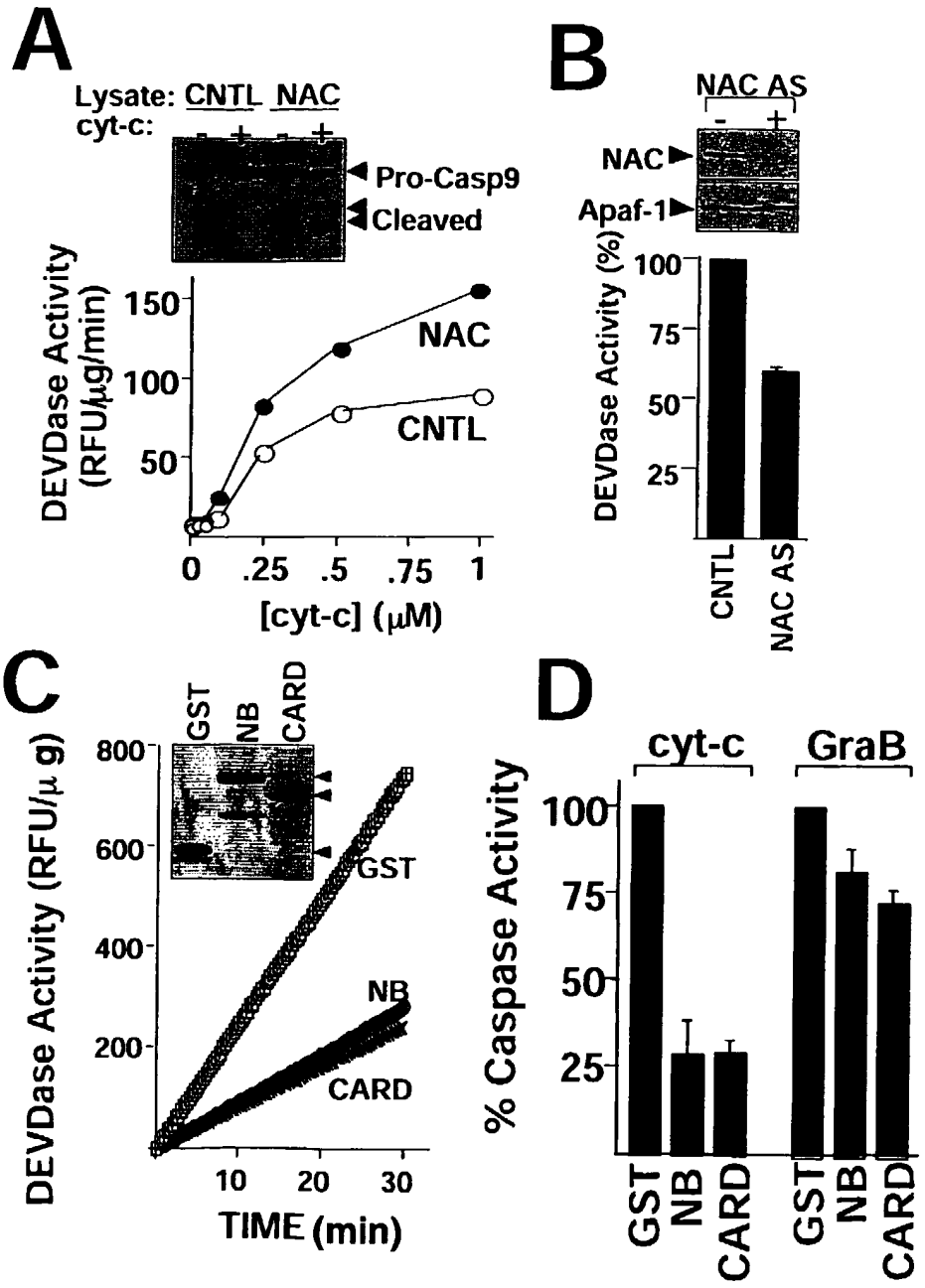

A
Lysate: CNTL NAC
cyt-c: - + - +
Pro-Casp9
Cleaved
DEVDase Activity (RFU/μg/min)
150
100
50
NAC
CNTL
0 .25 .5 .75 1
[cyt-c] (μM)
B
NAC AS
- +
NAC
Apaf-1
DEVDase Activity (%)
100
75
50
25
CNTL
NAC AS
C
GST NB CARD
DEVDase Activity (RFU/μ g)
800
600
400
200
GST
NB
CARD
10 20 30
TIME (min)
D
cyt-c
GraB
% Caspase Activity
100
75
50
25
GST NB CARD GST NB CARD

FIGURE 5

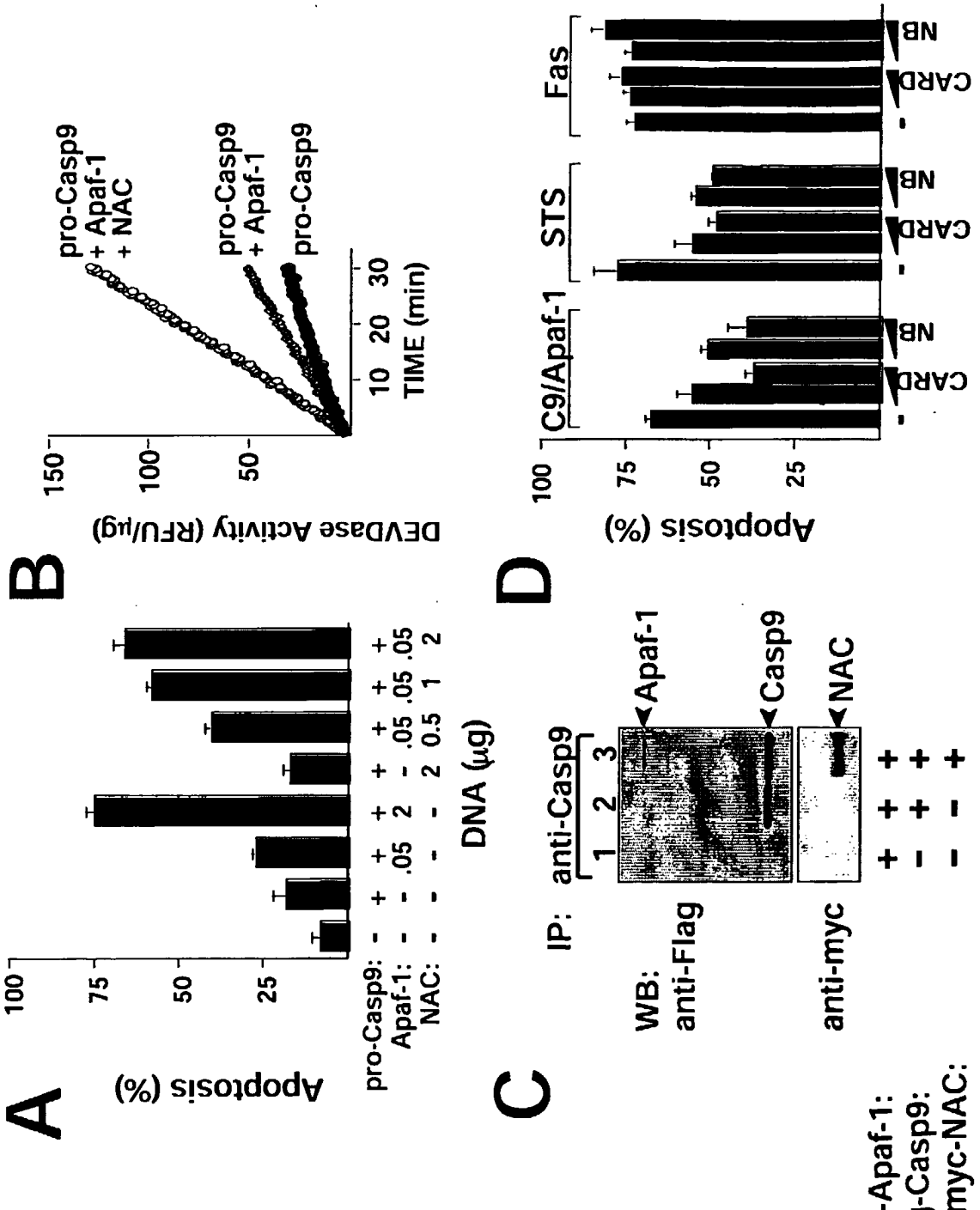

A
Apoptosis (%)
100
75
50
25
pro-Casp9: - + + + + + + + +
Apaf-1: - - .05 2 - .05 .05 .05
NAC: - - - - 2 0.5 1 2
DNA (μg)
B
DEVDase Activity (RFU/μg)
150
100
50
pro-Casp9 + Apaf-1 + NAC
pro-Casp9 + Apaf-1
pro-Casp9
10 20 30
TIME (min)
C
IP: anti-Casp9
1 2 3
Apaf-1
Casp9
NAC
WB: anti-Flag
anti-myc
Flag-Apaf-1: + + + +
Flag-Casp9: + + + -
myc-NAC: + - + -
D
Apoptosis (%)
100
75
50
25
C9/Apaf-1
STS
Fas
CARD
NB
- CARD
NB

FIGURE 6

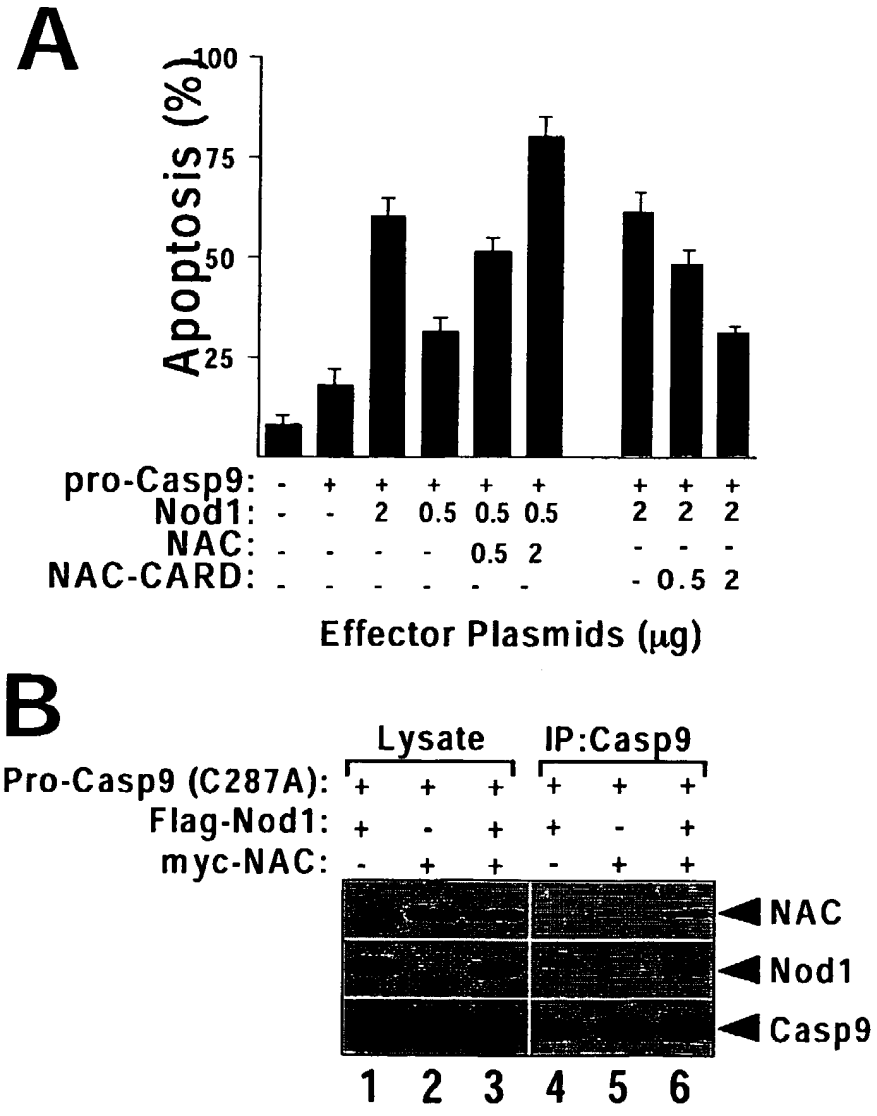

A
Apoptosis (%)
100
75
50
25
pro-Casp9: - + + + + + + + +
Nod1: - - 2 0.5 0.5 0.5 2 2 2
NAC: - - - - 0.5 2 - - -
NAC-CARD: - - - - - - - 0.5 2
Effector Plasmids (μg)
B
Lysate
IP:Casp9
Pro-Casp9 (C287A): + + + + + +
Flag-Nod1: + - + + - +
myc-NAC: - + + - + +
NAC
Nod1
Casp9
1 2 3 4 5 6

FIGURE 7

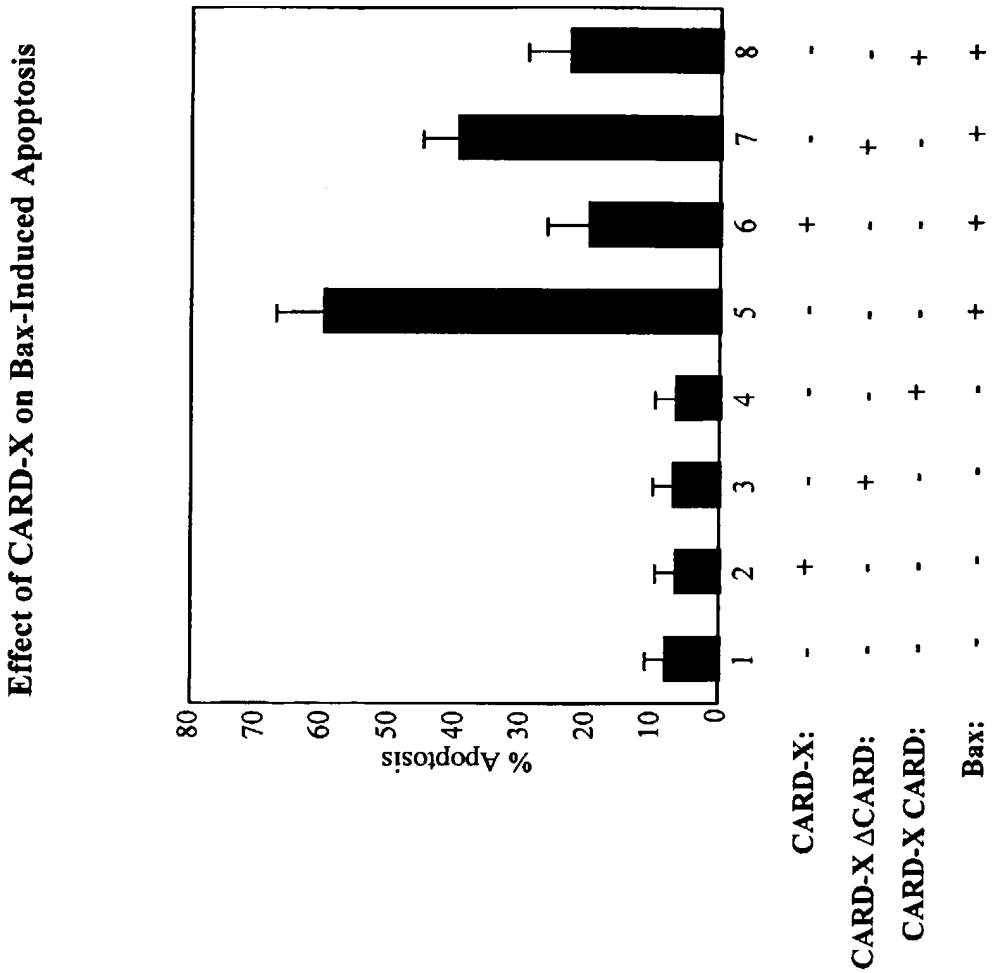
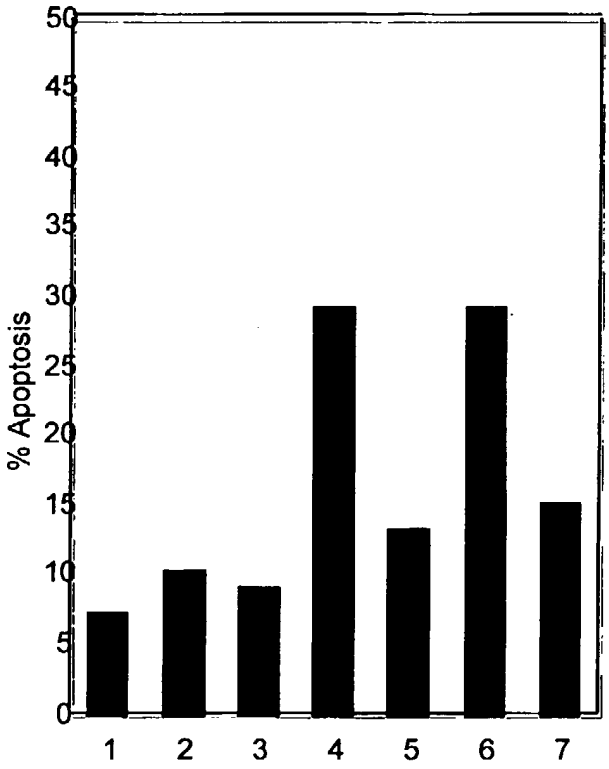

Effect of CARD-X on Bax-Induced Apoptosis
% Apoptosis
0
10
20
30
40
50
60
70
80
1
2
3
4
5
6
7
8
CARD-X:
CARD-X ΔCARD:
CARD-X CARD:
Bax:

FIGURE 8

Effect of CARD-X on Caspase9-induced Apoptosis



CARD-X:	-	-	-	-	+	-	-
CARD-X ΔCARD:	-	-	-	-	-	+	-
CARD-X CARD:	-	-	-	-	-	-	+
Caspase9:	-	+	-	+	+	+	+
Apaf-1:	-	-	+	+	+	+	+

FIGURE 9

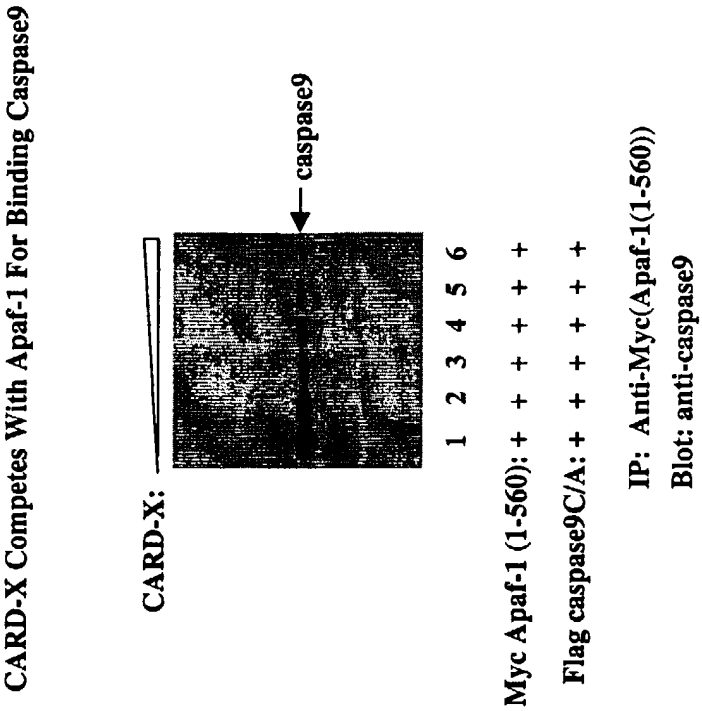

CARD-X Competes With Apaf-1 For Binding Caspase9
CARD-X:
caspase9
1 2 3 4 5 6
Myc Apaf-1 (1-560): + + + + + +
Flag caspase9C/A: + + + + + +
IP: Anti-Myc(Apaf-1(1-560))
Blot: anti-caspase9

FIGURE 10

SEQUENCE LISTING

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Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
            20                  25                  30

cac tcc agg agc tct tcg ggt gag aca ccc gct cag cca gag aag acg   144
His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
         35                  40                  45

agt ggc atg gag gtg gcc tcg tac ctg gtg gct cag tat ggg gag cag   192
Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
     50                  55                  60

cgg gcc tgg gac cta gcc ctc cat acc tgg gag cag atg ggg ctg agg   240
Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
 65                  70                  75                  80

tca ctg tgc gcc caa gcc cag gaa ggg gca ggc cac tct ccc tca ttc   288
Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                85                  90                  95

ccc tac agc cca agt gaa ccc cac ctg ggg tct ccc agc caa ccc acc   336
Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

tcc acc gca gtg cta atg ccc tgg atc cat gaa ttg ccg gcg ggg tgc   384
Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

acc cag ggc tca gag aga agg gtt ttg aga cag ctg cct gac aca tct   432
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Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

gga cgc cgc tgg aga gaa atc tct gcc tca ctc ctc tac caa gct ctt  480
Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

cca agc tcc cca gac cat gag tct cca agc cag gag tca ccc aac gcc  528
Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
                165                 170                 175

ccc aca tcc aca gca gtg ctg ggg agc tgg gga tcc cca cct cag ccc  576
Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

agc cta gca ccc aga gag cag gag gct cct ggg acc caa tgg cct ctg  624
Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

gat gaa acg tca gga att tac tac aca gaa atc aga gaa aga gag aga  672
Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

gag aaa tca gag aaa ggc agg ccc cca tgg gca gcg gtg gta gga acg  720
Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

ccc cca cag gcg cac acc agc cta cag ccc cac cac cac cca tgg gag  768
Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

cct tct gtg aga gag agc ctc tgt tcc aca tgg ccc tgg aaa aat gag  816
Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

gat ttt aac caa aaa ttc aca cag ctg cta ctt cta caa aga cct cac  864
Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
        275                 280                 285

ccc aga agc caa gat ccc ctg gtc aag aga agc tgg cct gat tat gtg  912
Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

gag gag aat cga gga cat tta att gag atc aga gac tta ttt ggc cca  960
Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

ggc ctg gat acc caa gaa cct cgc ata gtc ata ctg cag ggg gct gct 1008
Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

gga att ggg aag tca aca ctg gcc agg cag gtg aag gaa gcc tgg ggg 1056
Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

aga ggc cag ctg tat ggg gac cgc ttc cag cat gtc ttc tac ttc agc 1104
Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

tgc aga gag ctg gcc cag tcc aag gtg gtg agt ctc gct gag ctc atc 1152
Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380
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gga aaa gat ggg aca gcc act ccg gct ccc att aga cag atc ctg tct   1200
Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

agg cca gag cgg ctg ctc ttc atc ctc gat ggt gta gat gag cca gga   1248
Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415

tgg gtc ttg cag gag ccg agt tct gag ctc tgt ctg cac tgg agc cag   1296
Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

cca cag ccg gcg gat gca ctg ctg ggc agt ttg ctg ggg aaa act ata   1344
Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

ctt ccc gag gca tcc ttc ctg atc acg gct cgg acc aca gct ctg cag   1392
Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
    450                 455                 460

aac ctc att cct tct ttg gag cag gca cgt tgg gta gag gtc ctg ggg   1440
Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465                 470                 475                 480

ttc tct gag tcc agc agg aag gaa tat ttc tac aga tat ttc aca gat   1488
Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495

gaa agg caa gca att aga gcc ttt agg ttg gtc aaa tca aac aaa gag   1536
Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

ctc tgg gcc ctg tgt ctt gtg ccc tgg gtg tcc tgg ctg gcc tgc act   1584
Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

tgc ctg atg cag cag atg aag cgg aag gaa aaa ctc aca ctg act tcc   1632
Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

aag acc acc aca acc ctc tgt cta cat tac ctt gcc cag gct ctc caa   1680
Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

gct cag cca ttg gga ccc cag ctc aga gac ctc tgc tct ctg gct gct   1728
Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575

gag ggc atc tgg caa aaa aag acc ctt ttc agt cca gat gac ctc agg   1776
Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

aag cat ggg tta gat ggg gcc atc atc tcc acc ttc ttg aag atg ggt   1824
Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605

att ctt caa gag cac ccc atc cct ctg agc tac agc ttc att cac ctc   1872
Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

tgt ttc caa gag ttc ttt gca gca atg tcc tat gtc ttg gag gat gag   1920
Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
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625                 630                 635                 640

aag ggg aga ggt aaa cat tct aat tgc atc ata gat ttg gaa aag acg   1968
Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

cta gaa gca tat gga ata cat ggc ctg ttt ggg gca tca acc aca cgt   2016
Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
            660                 665                 670

ttc cta ttg ggc ctg tta agt gat gag ggg gag aga gag atg gag aac   2064
Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

atc ttt cac tgc cgg ctg tct cag ggg agg aac ctg atg cag tgg gtc   2112
Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

ccg tcc ctg cag ctg ctg ctg cag cca cac tct ctg gag tcc ctc cac   2160
Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

tgc ttg tac gag act cgg aac aaa acg ttc ctg aca caa gtg atg gcc   2208
Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

cat ttc gaa gaa atg ggc atg tgt gta gaa aca gac atg gag ctc tta   2256
His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

gtg tgc act ttc tgc att aaa ttc agc cgc cac gtg aag aag ctt cag   2304
Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

ctg att gag ggc agg cag cac aga tca aca tgg agc ccc acc atg gta   2352
Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

gtc ctg ttc agg tgg gtc cca gtc aca gat gcc tat tgg cag att ctc   2400
Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

ttc tcc gtc ctc aag gtc acc aga aac ctg aag gag ctg gac cta agt   2448
Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

gga aac tcg ctg agc cac tct gca gtg aag agt ctt tgt aag acc ctg   2496
Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

aga cgc cct cgc tgc ctc ctg gag acc ctg cgg ttg gct ggc tgt ggc   2544
Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

ctc aca gct gag gac tgc aag gac ctt gcc ttt ggg ctg aga gcc aac   2592
Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

cag acc ctg acc gag ctg gac ctg agc ttc aat gtg ctc acg gat gct   2640
Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880
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gga gcc aaa cac ctt tgc cag aga ctg aga cag ccg agc tgc aag cta   2688
Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

cag cga ctg cag ctg gtc agc tgt ggc ctc acg tct gac tgc tgc cag   2736
Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910

gac ctg gcc tct gtg ctt agt gcc agc ccc agc ctg aag gag cta gac   2784
Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

ctg cag cag aac aac ctg gat gac gtt ggc gtg cga ctg ctc tgt gag   2832
Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

ggg ctc agg cat cct gcc tgc aaa ctc ata cgc ctg ggg ctg gac cag   2880
Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Gly Leu Asp Gln
945                 950                 955                 960

aca act ctg agt gat gag atg agg cag gaa ctg agg gcc ctg gag cag   2928
Thr Thr Leu Ser Asp Glu Met Arg Gln Glu Leu Arg Ala Leu Glu Gln
                965                 970                 975

gag aaa cct cag ctg ctc atc ttc agc aga cgg aaa cca agt gtg atg   2976
Glu Lys Pro Gln Leu Leu Ile Phe Ser Arg Arg Lys Pro Ser Val Met
            980                 985                 990

acc cct act gag ggc ctg gat acg gga gag atg agt aat agc aca tcc   3024
Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser Thr Ser
        995                1000                1005

tca ctc aag cgg cag aga ctc gga tca gag agg gcg gct tcc cat gtt   3072
Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser His Val
   1010                1015                1020

gct cag gct aat ctc aaa ctc ctg gac gtg agc aag atc ttc cca att   3120
Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe Pro Ile
1025                1030                1035                1040

gct gag att gca gag gaa agc tcc cca gag gta gta ccg gtg gaa ctc   3168
Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val Glu Leu
               1045                1050                1055

ttg tgc gtg cct tct cct gcc tct caa ggg gac ctg cat acg aag cct   3216
Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr Lys Pro
           1060                1065                1070

ttg ggg act gac gat gac ttc tgg ggc ccc acg ggg cct gtg gct act   3264
Leu Gly Thr Asp Asp Asp Phe Trp Gly Pro Thr Gly Pro Val Ala Thr
       1075                1080                1085

gag gta gtt gac aaa gaa aag aac ttg tac cga gtt cac ttc cct gta   3312
Glu Val Val Asp Lys Glu Lys Asn Leu Tyr Arg Val His Phe Pro Val
   1090                1095                1100

gct ggc tcc tac cgc tgg ccc aac acg ggt ctc tgc ttt gtg atg aga   3360
Ala Gly Ser Tyr Arg Trp Pro Asn Thr Gly Leu Cys Phe Val Met Arg
1105                1110                1115                1120

gaa gcg gtg acc gtt gag att gaa ttc tgt gtg tgg gac cag ttc ctg   3408
Glu Ala Val Thr Val Glu Ile Glu Phe Cys Val Trp Asp Gln Phe Leu
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                1125                1130                1135

ggt gag atc aac cca cag cac agc tgg atg gtg gca ggg cct ctg ctg   3456
Gly Glu Ile Asn Pro Gln His Ser Trp Met Val Ala Gly Pro Leu Leu
            1140                1145                1150

gac atc aag gct gag cct gga gct gtg gaa gct gtg cac ctc cct cac   3504
Asp Ile Lys Ala Glu Pro Gly Ala Val Glu Ala Val His Leu Pro His
        1155                1160                1165

ttt gtg gct ctc caa ggg ggc cat gtg gac aca tcc ctg ttc caa atg   3552
Phe Val Ala Leu Gln Gly Gly His Val Asp Thr Ser Leu Phe Gln Met
    1170                1175                1180

gcc cac ttt aaa gag gag ggg atg ctc ctg gag aag cca gcc agg gtg   3600
Ala His Phe Lys Glu Glu Gly Met Leu Leu Glu Lys Pro Ala Arg Val
1185                1190                1195                1200

gag ctg cat cac ata gtt ctg gaa aac ccc agc ttc tcc ccc ttg gga   3648
Glu Leu His His Ile Val Leu Glu Asn Pro Ser Phe Ser Pro Leu Gly
                1205                1210                1215

gtc ctc ctg aaa atg atc cat aat gcc ctg cgc ttc att ccc gtc acc   3696
Val Leu Leu Lys Met Ile His Asn Ala Leu Arg Phe Ile Pro Val Thr
            1220                1225                1230

tct gtg gtg ttg ctt tac cac cgc gtc cat cct gag gaa gtc acc ttc   3744
Ser Val Val Leu Leu Tyr His Arg Val His Pro Glu Glu Val Thr Phe
        1235                1240                1245

cac ctc tac ctg atc cca agt gac tgc tcc att cgg aag gcc ata gat   3792
His Leu Tyr Leu Ile Pro Ser Asp Cys Ser Ile Arg Lys Ala Ile Asp
    1250                1255                1260

gat cta gaa atg aaa ttc cag ttt gtg cga atc cac aag cca ccc ccg   3840
Asp Leu Glu Met Lys Phe Gln Phe Val Arg Ile His Lys Pro Pro Pro
1265                1270                1275                1280

ctg acc cca ctt tat atg ggc tgt cgt tac act gtg tct ggg tct ggt   3888
Leu Thr Pro Leu Tyr Met Gly Cys Arg Tyr Thr Val Ser Gly Ser Gly
                1285                1290                1295

tca ggg atg ctg gaa ata ctc ccc aag gaa ctg gag ctc tgc tat cga   3936
Ser Gly Met Leu Glu Ile Leu Pro Lys Glu Leu Glu Leu Cys Tyr Arg
            1300                1305                1310

agc cct gga gaa gac cag ctg ttc tcg gag ttc tac gtt ggc cac ttg   3984
Ser Pro Gly Glu Asp Gln Leu Phe Ser Glu Phe Tyr Val Gly His Leu
        1315                1320                1325

gga tca ggg atc agg ctg caa gtg aaa gac aag aaa gat gag act ctg   4032
Gly Ser Gly Ile Arg Leu Gln Val Lys Asp Lys Lys Asp Glu Thr Leu
    1330                1335                1340

gtg tgg gag gcc ttg gtg aaa cca gga gat ctc atg cct gca act act   4080
Val Trp Glu Ala Leu Val Lys Pro Gly Asp Leu Met Pro Ala Thr Thr
1345                1350                1355                1360

ctg atc cct cca gcc cgc ata gcc gta cct tca cct ctg gat gcc ccg   4128
Leu Ile Pro Pro Ala Arg Ile Ala Val Pro Ser Pro Leu Asp Ala Pro
                1365                1370                1375
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cag ttg ctg cac ttt gtg gac cag tat cga gag cag ctg ata gcc cga   4176
Gln Leu Leu His Phe Val Asp Gln Tyr Arg Glu Gln Leu Ile Ala Arg
          1380                1385                1390

gtg aca tcg gtg gag gtt gtc ttg gac aaa ctg cat gga cag gtg ctg   4224
Val Thr Ser Val Glu Val Val Leu Asp Lys Leu His Gly Gln Val Leu
       1395                1400                1405

agc cag gag cag tac gag agg gtg ctg gct gag aac acg agg ccc agc   4272
Ser Gln Glu Gln Tyr Glu Arg Val Leu Ala Glu Asn Thr Arg Pro Ser
   1410                1415                1420

cag atg cgg aag ctg ttc agc ttg agc cag tcc tgg gac cgg aag tgc   4320
Gln Met Arg Lys Leu Phe Ser Leu Ser Gln Ser Trp Asp Arg Lys Cys
1425                1430                1435                1440

aaa gat gga ctc tac caa gcc ctg aag gag acc cat cct cac ctc att   4368
Lys Asp Gly Leu Tyr Gln Ala Leu Lys Glu Thr His Pro His Leu Ile
               1445                1450                1455

atg gaa ctc tgg gag aag ggc agc aaa aag gga ctc ctg cca ctc agc   4416
Met Glu Leu Trp Glu Lys Gly Ser Lys Lys Gly Leu Leu Pro Leu Ser
          1460                1465                1470

agc tga                                                          4422
Ser
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<210> 2
<211> 1473
<212> PRT
<213> Homo sapiens

<400> 2

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Met Ala Gly Gly Ala Trp Gly Arg Leu Ala Cys Tyr Leu Glu Phe Leu
  1               5                  10                  15

Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
             20                  25                  30

His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
         35                  40                  45

Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
     50                  55                  60

Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
 65                  70                  75                  80

Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                 85                  90                  95

Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
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145                 150                 155                 160

Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
                165                 170                 175

Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
        275                 280                 285

Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380

Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415

Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
    450                 455                 460

Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465                 470                 475                 480
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Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495

Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575

Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605

Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625                 630                 635                 640

Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
            660                 665                 670

Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815
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Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910

Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Gly Leu Asp Gln
945                 950                 955                 960

Thr Thr Leu Ser Asp Glu Met Arg Gln Glu Leu Arg Ala Leu Glu Gln
                965                 970                 975

Glu Lys Pro Gln Leu Leu Ile Phe Ser Arg Arg Lys Pro Ser Val Met
            980                 985                 990

Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser Thr Ser
        995                1000                1005

Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser His Val
   1010                1015                1020

Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe Pro Ile
1025                1030                1035                1040

Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val Glu Leu
               1045                1050                1055

Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr Lys Pro
           1060                1065                1070

Leu Gly Thr Asp Asp Asp Phe Trp Gly Pro Thr Gly Pro Val Ala Thr
       1075                1080                1085

Glu Val Val Asp Lys Glu Lys Asn Leu Tyr Arg Val His Phe Pro Val
   1090                1095                1100

Ala Gly Ser Tyr Arg Trp Pro Asn Thr Gly Leu Cys Phe Val Met Arg
1105                1110                1115                1120

Glu Ala Val Thr Val Glu Ile Glu Phe Cys Val Trp Asp Gln Phe Leu
               1125                1130                1135

Gly Glu Ile Asn Pro Gln His Ser Trp Met Val Ala Gly Pro Leu Leu
           1140                1145                1150
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Asp Ile Lys Ala Glu Pro Gly Ala Val Glu Ala Val His Leu Pro His
1155 1160 1165

Phe Val Ala Leu Gln Gly Gly His Val Asp Thr Ser Leu Phe Gln Met
1170 1175 1180

Ala His Phe Lys Glu Glu Gly Met Leu Leu Glu Lys Pro Ala Arg Val
1185 1190 1195 1200

Glu Leu His His Ile Val Leu Glu Asn Pro Ser Phe Ser Pro Leu Gly
1205 1210 1215

Val Leu Leu Lys Met Ile His Asn Ala Leu Arg Phe Ile Pro Val Thr
1220 1225 1230

Ser Val Val Leu Leu Tyr His Arg Val His Pro Glu Glu Val Thr Phe
1235 1240 1245

His Leu Tyr Leu Ile Pro Ser Asp Cys Ser Ile Arg Lys Ala Ile Asp
1250 1255 1260

Asp Leu Glu Met Lys Phe Gln Phe Val Arg Ile His Lys Pro Pro Pro
1265 1270 1275 1280

Leu Thr Pro Leu Tyr Met Gly Cys Arg Tyr Thr Val Ser Gly Ser Gly
1285 1290 1295

Ser Gly Met Leu Glu Ile Leu Pro Lys Glu Leu Glu Leu Cys Tyr Arg
1300 1305 1310

Ser Pro Gly Glu Asp Gln Leu Phe Ser Glu Phe Tyr Val Gly His Leu
1315 1320 1325

Gly Ser Gly Ile Arg Leu Gln Val Lys Asp Lys Lys Asp Glu Thr Leu
1330 1335 1340

Val Trp Glu Ala Leu Val Lys Pro Gly Asp Leu Met Pro Ala Thr Thr
1345 1350 1355 1360

Leu Ile Pro Pro Ala Arg Ile Ala Val Pro Ser Pro Leu Asp Ala Pro
1365 1370 1375

Gln Leu Leu His Phe Val Asp Gln Tyr Arg Glu Gln Leu Ile Ala Arg
1380 1385 1390

Val Thr Ser Val Glu Val Val Leu Asp Lys Leu His Gly Gln Val Leu
1395 1400 1405

Ser Gln Glu Gln Tyr Glu Arg Val Leu Ala Glu Asn Thr Arg Pro Ser
1410 1415 1420

Gln Met Arg Lys Leu Phe Ser Leu Ser Gln Ser Trp Asp Arg Lys Cys
1425 1430 1435 1440

Lys Asp Gly Leu Tyr Gln Ala Leu Lys Glu Thr His Pro His Leu Ile
1445 1450 1455

Met Glu Leu Trp Glu Lys Gly Ser Lys Lys Gly Leu Leu Pro Leu Ser
1460 1465 1470

Ser

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Met Ala Gly Gly Ala Trp Gly Arg Leu Ala Cys Tyr Leu Glu Phe Leu
  1               5                  10                  15

aag aag gag gag ctg aag gag ttc cag ctt ctg ctc gcc aat aaa gcg   96
Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
             20                  25                  30

cac tcc agg agc tct tcg ggt gag aca ccc gct cag cca gag aag acg   144
His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
         35                  40                  45

agt ggc atg gag gtg gcc tcg tac ctg gtg gct cag tat ggg gag cag   192
Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
     50                  55                  60

cgg gcc tgg gac cta gcc ctc cat acc tgg gag cag atg ggg ctg agg   240
Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
 65                  70                  75                  80

tca ctg tgc gcc caa gcc cag gaa ggg gca ggc cac tct ccc tca ttc   288
Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                 85                  90                  95

ccc tac agc cca agt gaa ccc cac ctg ggg tct ccc agc caa ccc acc   336
Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

tcc acc gca gtg cta atg ccc tgg atc cat gaa ttg ccg gcg ggg tgc   384
Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

acc cag ggc tca gag aga agg gtt ttg aga cag ctg cct gac aca tct   432
Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

gga cgc cgc tgg aga gaa atc tct gcc tca ctc ctc tac caa gct ctt   480
Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

cca agc tcc cca gac cat gag tct cca agc cag gag tca ccc aac gcc   528
Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
                165                 170                 175

ccc aca tcc aca gca gtg ctg ggg agc tgg gga tcc cca cct cag ccc   576
Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

agc cta gca ccc aga gag cag gag gct cct ggg acc caa tgg cct ctg   624
Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205
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gat gaa acg tca gga att tac tac aca gaa atc aga gaa aga gag aga   672
Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

gag aaa tca gag aaa ggc agg ccc cca tgg gca gcg gtg gta gga acg   720
Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

ccc cca cag gcg cac acc agc cta cag ccc cac cac cac cca tgg gag   768
Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

cct tct gtg aga gag agc ctc tgt tcc aca tgg ccc tgg aaa aat gag   816
Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

gat ttt aac caa aaa ttc aca cag ctg cta ctt cta caa aga cct cac   864
Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
        275                 280                 285

ccc aga agc caa gat ccc ctg gtc aag aga agc tgg cct gat tat gtg   912
Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

gag gag aat cga gga cat tta att gag atc aga gac tta ttt ggc cca   960
Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

ggc ctg gat acc caa gaa cct cgc ata gtc ata ctg cag ggg gct gct  1008
Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

gga att ggg aag tca aca ctg gcc agg cag gtg aag gaa gcc tgg ggg  1056
Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

aga ggc cag ctg tat ggg gac cgc ttc cag cat gtc ttc tac ttc agc  1104
Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

tgc aga gag ctg gcc cag tcc aag gtg gtg agt ctc gct gag ctc atc  1152
Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380

gga aaa gat ggg aca gcc act ccg gct ccc att aga cag atc ctg tct  1200
Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

agg cca gag cgg ctg ctc ttc atc ctc gat ggt gta gat gag cca gga  1248
Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415

tgg gtc ttg cag gag ccg agt tct gag ctc tgt ctg cac tgg agc cag  1296
Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

cca cag ccg gcg gat gca ctg ctg ggc agt ttg ctg ggg aaa act ata  1344
Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

ctt ccc gag gca tcc ttc ctg atc acg gct cgg acc aca gct ctg cag  1392
Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
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    450                 455                 460

aac ctc att cct tct ttg gag cag gca cgt tgg gta gag gtc ctg ggg   1440
Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465                 470                 475                 480

ttc tct gag tcc agc agg aag gaa tat ttc tac aga tat ttc aca gat   1488
Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495

gaa agg caa gca att aga gcc ttt agg ttg gtc aaa tca aac aaa gag   1536
Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

ctc tgg gcc ctg tgt ctt gtg ccc tgg gtg tcc tgg ctg gcc tgc act   1584
Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

tgc ctg atg cag cag atg aag cgg aag gaa aaa ctc aca ctg act tcc   1632
Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

aag acc acc aca acc ctc tgt cta cat tac ctt gcc cag gct ctc caa   1680
Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

gct cag cca ttg gga ccc cag ctc aga gac ctc tgc tct ctg gct gct   1728
Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575

gag ggc atc tgg caa aaa aag acc ctt ttc agt cca gat gac ctc agg   1776
Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

aag cat ggg tta gat ggg gcc atc atc tcc acc ttc ttg aag atg ggt   1824
Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605

att ctt caa gag cac ccc atc cct ctg agc tac agc ttc att cac ctc   1872
Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

tgt ttc caa gag ttc ttt gca gca atg tcc tat gtc ttg gag gat gag   1920
Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625                 630                 635                 640

aag ggg aga ggt aaa cat tct aat tgc atc ata gat ttg gaa aag acg   1968
Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

cta gaa gca tat gga ata cat ggc ctg ttt ggg gca tca acc aca cgt   2016
Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
            660                 665                 670

ttc cta ttg ggc ctg tta agt gat gag ggg gag aga gag atg gag aac   2064
Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

atc ttt cac tgc cgg ctg tct cag ggg agg aac ctg atg cag tgg gtc   2112
Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700
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ccg tcc ctg cag ctg ctg ctg cag cca cac tct ctg gag tcc ctc cac   2160
Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

tgc ttg tac gag act cgg aac aaa acg ttc ctg aca caa gtg atg gcc   2208
Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

cat ttc gaa gaa atg ggc atg tgt gta gaa aca gac atg gag ctc tta   2256
His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

gtg tgc act ttc tgc att aaa ttc agc cgc cac gtg aag aag ctt cag   2304
Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

ctg att gag ggc agg cag cac aga tca aca tgg agc ccc acc atg gta   2352
Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

gtc ctg ttc agg tgg gtc cca gtc aca gat gcc tat tgg cag att ctc   2400
Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

ttc tcc gtc ctc aag gtc acc aga aac ctg aag gag ctg gac cta agt   2448
Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

gga aac tcg ctg agc cac tct gca gtg aag agt ctt tgt aag acc ctg   2496
Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

aga cgc cct cgc tgc ctc ctg gag acc ctg cgg ttg gct ggc tgt ggc   2544
Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

ctc aca gct gag gac tgc aag gac ctt gcc ttt ggg ctg aga gcc aac   2592
Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

cag acc ctg acc gag ctg gac ctg agc ttc aat gtg ctc acg gat gct   2640
Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

gga gcc aaa cac ctt tgc cag aga ctg aga cag ccg agc tgc aag cta   2688
Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

cag cga ctg cag ctg gtc agc tgt ggc ctc acg tct gac tgc tgc cag   2736
Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910

gac ctg gcc tct gtg ctt agt gcc agc ccc agc ctg aag gag cta gac   2784
Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

ctg cag cag aac aac ctg gat gac gtt ggc gtg cga ctg ctc tgt gag   2832
Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

ggg ctc agg cat cct gcc tgc aaa ctc ata cgc ctg aaa cca agt gtg   2880
Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Lys Pro Ser Val
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945                 950                 955                 960

atg acc cct act gag ggc ctg gat acg gga gag atg agt aat agc aca   2928
Met Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser Thr
                965                 970                 975

tcc tca ctc aag cgg cag aga ctc gga tca gag agg gcg gct tcc cat   2976
Ser Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser His
            980                 985                 990

gtt gct cag gct aat ctc aaa ctc ctg gac gtg agc aag atc ttc cca   3024
Val Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe Pro
        995                1000                1005

att gct gag att gca gag gaa agc tcc cca gag gta gta ccg gtg gaa   3072
Ile Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val Glu
   1010                1015                1020

ctc ttg tgc gtg cct tct cct gcc tct caa ggg gac ctg cat acg aag   3120
Leu Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr Lys
1025                1030                1035                1040

cct ttg ggg act gac gat gac ttc tgg ggc ccc acg ggg cct gtg gct   3168
Pro Leu Gly Thr Asp Asp Asp Phe Trp Gly Pro Thr Gly Pro Val Ala
               1045                1050                1055

act gag gta gtt gac aaa gaa aag aac ttg tac cga gtt cac ttc cct   3216
Thr Glu Val Val Asp Lys Glu Lys Asn Leu Tyr Arg Val His Phe Pro
           1060                1065                1070

gta gct ggc tcc tac cgc tgg ccc aac acg ggt ctc tgc ttt gtg atg   3264
Val Ala Gly Ser Tyr Arg Trp Pro Asn Thr Gly Leu Cys Phe Val Met
       1075                1080                1085

aga gaa gcg gtg acc gtt gag att gaa ttc tgt gtg tgg gac cag ttc   3312
Arg Glu Ala Val Thr Val Glu Ile Glu Phe Cys Val Trp Asp Gln Phe
   1090                1095                1100

ctg ggt gag atc aac cca cag cac agc tgg atg gtg gca ggg cct ctg   3360
Leu Gly Glu Ile Asn Pro Gln His Ser Trp Met Val Ala Gly Pro Leu
1105                1110                1115                1120

ctg gac atc aag gct gag cct gga gct gtg gaa gct gtg cac ctc cct   3408
Leu Asp Ile Lys Ala Glu Pro Gly Ala Val Glu Ala Val His Leu Pro
               1125                1130                1135

cac ttt gtg gct ctc caa ggg ggc cat gtg gac aca tcc ctg ttc caa   3456
His Phe Val Ala Leu Gln Gly Gly His Val Asp Thr Ser Leu Phe Gln
           1140                1145                1150

atg gcc cac ttt aaa gag gag ggg atg ctc ctg gag aag cca gcc agg   3504
Met Ala His Phe Lys Glu Glu Gly Met Leu Leu Glu Lys Pro Ala Arg
       1155                1160                1165

gtg gag ctg cat cac ata gtt ctg gaa aac ccc agc ttc tcc ccc ttg   3552
Val Glu Leu His His Ile Val Leu Glu Asn Pro Ser Phe Ser Pro Leu
   1170                1175                1180

gga gtc ctc ctg aaa atg atc cat aat gcc ctg cgc ttc att ccc gtc   3600
Gly Val Leu Leu Lys Met Ile His Asn Ala Leu Arg Phe Ile Pro Val
1185                1190                1195                1200
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acc tct gtg gtg ttg ctt tac cac cgc gtc cat cct gag gaa gtc acc   3648
Thr Ser Val Val Leu Leu Tyr His Arg Val His Pro Glu Glu Val Thr
              1205                1210                1215

ttc cac ctc tac ctg atc cca agt gac tgc tcc att cgg gaa ctg gag   3696
Phe His Leu Tyr Leu Ile Pro Ser Asp Cys Ser Ile Arg Glu Leu Glu
          1220                1225                1230

ctc tgc tat cga agc cct gga gaa gac cag ctg ttc tcg gag ttc tac   3744
Leu Cys Tyr Arg Ser Pro Gly Glu Asp Gln Leu Phe Ser Glu Phe Tyr
       1235                1240                1245

gtt ggc cac ttg gga tca ggg atc agg ctg caa gtg aaa gac aag aaa   3792
Val Gly His Leu Gly Ser Gly Ile Arg Leu Gln Val Lys Asp Lys Lys
   1250                1255                1260

gat gag act ctg gtg tgg gag gcc ttg gtg aaa cca gga gat ctc atg   3840
Asp Glu Thr Leu Val Trp Glu Ala Leu Val Lys Pro Gly Asp Leu Met
1265                1270                1275                1280

cct gca act act ctg atc cct cca gcc cgc ata gcc gta cct tca cct   3888
Pro Ala Thr Thr Leu Ile Pro Pro Ala Arg Ile Ala Val Pro Ser Pro
              1285                1290                1295

ctg gat gcc ccg cag ttg ctg cac ttt gtg gac cag tat cga gag cag   3936
Leu Asp Ala Pro Gln Leu Leu His Phe Val Asp Gln Tyr Arg Glu Gln
          1300                1305                1310

ctg ata gcc cga gtg aca tcg gtg gag gtt gtc ttg gac aaa ctg cat   3984
Leu Ile Ala Arg Val Thr Ser Val Glu Val Val Leu Asp Lys Leu His
       1315                1320                1325

gga cag gtg ctg agc cag gag cag tac gag agg gtg ctg gct gag aac   4032
Gly Gln Val Leu Ser Gln Glu Gln Tyr Glu Arg Val Leu Ala Glu Asn
   1330                1335                1340

acg agg ccc agc cag atg cgg aag ctg ttc agc ttg agc cag tcc tgg   4080
Thr Arg Pro Ser Gln Met Arg Lys Leu Phe Ser Leu Ser Gln Ser Trp
1345                1350                1355                1360

gac cgg aag tgc aaa gat gga ctc tac caa gcc ctg aag gag acc cat   4128
Asp Arg Lys Cys Lys Asp Gly Leu Tyr Gln Ala Leu Lys Glu Thr His
              1365                1370                1375

cct cac ctc att atg gaa ctc tgg gag aag ggc agc aaa aag gga ctc   4176
Pro His Leu Ile Met Glu Leu Trp Glu Lys Gly Ser Lys Lys Gly Leu
          1380                1385                1390

ctg cca ctc agc agc tga                                          4194
Leu Pro Leu Ser Ser
       1395
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  1               5                  10                  15
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Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
            20                  25                  30

His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
        35                  40                  45

Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
    50                  55                  60

Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
65                  70                  75                  80

Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                85                  90                  95

Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
                165                 170                 175

Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
        275                 280                 285

Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350
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Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
355 360 365

Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
370 375 380

Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385 390 395 400

Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
405 410 415

Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
420 425 430

Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
435 440 445

Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
450 455 460

Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465 470 475 480

Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
485 490 495

Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
500 505 510

Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
515 520 525

Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
530 535 540

Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545 550 555 560

Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
565 570 575

Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
580 585 590

Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
595 600 605

Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
610 615 620

Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625 630 635 640

Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
645 650 655

Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
660 665 670

Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
675 680 685

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Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910

Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Lys Pro Ser Val
945                 950                 955                 960

Met Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser Thr
                965                 970                 975

Ser Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser His
            980                 985                 990

Val Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe Pro
        995                1000                1005

Ile Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val Glu
   1010                1015                1020
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Leu Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr Lys
1025                1030                1035                1040

Pro Leu Gly Thr Asp Asp Asp Phe Trp Gly Pro Thr Gly Pro Val Ala
                1045                1050                1055

Thr Glu Val Val Asp Lys Glu Lys Asn Leu Tyr Arg Val His Phe Pro
            1060                1065                1070

Val Ala Gly Ser Tyr Arg Trp Pro Asn Thr Gly Leu Cys Phe Val Met
        1075                1080                1085

Arg Glu Ala Val Thr Val Glu Ile Glu Phe Cys Val Trp Asp Gln Phe
    1090                1095                1100

Leu Gly Glu Ile Asn Pro Gln His Ser Trp Met Val Ala Gly Pro Leu
1105                1110                1115                1120

Leu Asp Ile Lys Ala Glu Pro Gly Ala Val Glu Ala Val His Leu Pro
                1125                1130                1135

His Phe Val Ala Leu Gln Gly Gly His Val Asp Thr Ser Leu Phe Gln
            1140                1145                1150

Met Ala His Phe Lys Glu Glu Gly Met Leu Leu Glu Lys Pro Ala Arg
        1155                1160                1165

Val Glu Leu His His Ile Val Leu Glu Asn Pro Ser Phe Ser Pro Leu
    1170                1175                1180

Gly Val Leu Leu Lys Met Ile His Asn Ala Leu Arg Phe Ile Pro Val
1185                1190                1195                1200

Thr Ser Val Val Leu Leu Tyr His Arg Val His Pro Glu Glu Val Thr
                1205                1210                1215

Phe His Leu Tyr Leu Ile Pro Ser Asp Cys Ser Ile Arg Glu Leu Glu
            1220                1225                1230

Leu Cys Tyr Arg Ser Pro Gly Glu Asp Gln Leu Phe Ser Glu Phe Tyr
        1235                1240                1245

Val Gly His Leu Gly Ser Gly Ile Arg Leu Gln Val Lys Asp Lys Lys
    1250                1255                1260

Asp Glu Thr Leu Val Trp Glu Ala Leu Val Lys Pro Gly Asp Leu Met
1265                1270                1275                1280

Pro Ala Thr Thr Leu Ile Pro Pro Ala Arg Ile Ala Val Pro Ser Pro
                1285                1290                1295

Leu Asp Ala Pro Gln Leu Leu His Phe Val Asp Gln Tyr Arg Glu Gln
            1300                1305                1310

Leu Ile Ala Arg Val Thr Ser Val Glu Val Val Leu Asp Lys Leu His
        1315                1320                1325

Gly Gln Val Leu Ser Gln Glu Gln Tyr Glu Arg Val Leu Ala Glu Asn
    1330                1335                1340

Thr Arg Pro Ser Gln Met Arg Lys Leu Phe Ser Leu Ser Gln Ser Trp
1345                1350                1355                1360
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Asp Arg Lys Cys Lys Asp Gly Leu Tyr Gln Ala Leu Lys Glu Thr His
                1365                1370                1375

Pro His Leu Ile Met Glu Leu Trp Glu Lys Gly Ser Lys Lys Gly Leu
            1380                1385                1390

Leu Pro Leu Ser Ser
        1395
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Met Ala Gly Gly Ala Trp Gly Arg Leu Ala Cys Tyr Leu Glu Phe Leu
  1               5                  10                  15

aag aag gag gag ctg aag gag ttc cag ctt ctg ctc gcc aat aaa gcg   96
Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
             20                  25                  30

cac tcc agg agc tct tcg ggt gag aca ccc gct cag cca gag aag acg   144
His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
         35                  40                  45

agt ggc atg gag gtg gcc tcg tac ctg gtg gct cag tat ggg gag cag   192
Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
     50                  55                  60

cgg gcc tgg gac cta gcc ctc cat acc tgg gag cag atg ggg ctg agg   240
Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
 65                  70                  75                  80

tca ctg tgc gcc caa gcc cag gaa ggg gca ggc cac tct ccc tca ttc   288
Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                 85                  90                  95

ccc tac agc cca agt gaa ccc cac ctg ggg tct ccc agc caa ccc acc   336
Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

tcc acc gca gtg cta atg ccc tgg atc cat gaa ttg ccg gcg ggg tgc   384
Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

acc cag ggc tca gag aga agg gtt ttg aga cag ctg cct gac aca tct   432
Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

gga cgc cgc tgg aga gaa atc tct gcc tca ctc ctc tac caa gct ctt   480
Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

cca agc tcc cca gac cat gag tct cca agc cag gag tca ccc aac gcc   528
Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
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                165                 170                 175

ccc aca tcc aca gca gtg ctg ggg agc tgg gga tcc cca cct cag ccc   576
Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

agc cta gca ccc aga gag cag gag gct cct ggg acc caa tgg cct ctg   624
Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

gat gaa acg tca gga att tac tac aca gaa atc aga gaa aga gag aga   672
Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

gag aaa tca gag aaa ggc agg ccc cca tgg gca gcg gtg gta gga acg   720
Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

ccc cca cag gcg cac acc agc cta cag ccc cac cac cac cca tgg gag   768
Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

cct tct gtg aga gag agc ctc tgt tcc aca tgg ccc tgg aaa aat gag   816
Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

gat ttt aac caa aaa ttc aca cag ctg cta ctt cta caa aga cct cac   864
Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
        275                 280                 285

ccc aga agc caa gat ccc ctg gtc aag aga agc tgg cct gat tat gtg   912
Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

gag gag aat cga gga cat tta att gag atc aga gac tta ttt ggc cca   960
Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

ggc ctg gat acc caa gaa cct cgc ata gtc ata ctg cag ggg gct gct  1008
Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

gga att ggg aag tca aca ctg gcc agg cag gtg aag gaa gcc tgg ggg  1056
Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

aga ggc cag ctg tat ggg gac cgc ttc cag cat gtc ttc tac ttc agc  1104
Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

tgc aga gag ctg gcc cag tcc aag gtg gtg agt ctc gct gag ctc atc  1152
Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380

gga aaa gat ggg aca gcc act ccg gct ccc att aga cag atc ctg tct  1200
Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

agg cca gag cgg ctg ctc ttc atc ctc gat ggt gta gat gag cca gga  1248
Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415
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tgg gtc ttg cag gag ccg agt tct gag ctc tgt ctg cac tgg agc cag   1296
Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

cca cag ccg gcg gat gca ctg ctg ggc agt ttg ctg ggg aaa act ata   1344
Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

ctt ccc gag gca tcc ttc ctg atc acg gct cgg acc aca gct ctg cag   1392
Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
    450                 455                 460

aac ctc att cct tct ttg gag cag gca cgt tgg gta gag gtc ctg ggg   1440
Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465                 470                 475                 480

ttc tct gag tcc agc agg aag gaa tat ttc tac aga tat ttc aca gat   1488
Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495

gaa agg caa gca att aga gcc ttt agg ttg gtc aaa tca aac aaa gag   1536
Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

ctc tgg gcc ctg tgt ctt gtg ccc tgg gtg tcc tgg ctg gcc tgc act   1584
Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

tgc ctg atg cag cag atg aag cgg aag gaa aaa ctc aca ctg act tcc   1632
Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

aag acc acc aca acc ctc tgt cta cat tac ctt gcc cag gct ctc caa   1680
Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

gct cag cca ttg gga ccc cag ctc aga gac ctc tgc tct ctg gct gct   1728
Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575

gag ggc atc tgg caa aaa aag acc ctt ttc agt cca gat gac ctc agg   1776
Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

aag cat ggg tta gat ggg gcc atc atc tcc acc ttc ttg aag atg ggt   1824
Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605

att ctt caa gag cac ccc atc cct ctg agc tac agc ttc att cac ctc   1872
Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

tgt ttc caa gag ttc ttt gca gca atg tcc tat gtc ttg gag gat gag   1920
Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625                 630                 635                 640

aag ggg aga ggt aaa cat tct aat tgc atc ata gat ttg gaa aag acg   1968
Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

cta gaa gca tat gga ata cat ggc ctg ttt ggg gca tca acc aca cgt   2016
Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
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            660                 665                 670

ttc cta ttg ggc ctg tta agt gat gag ggg gag aga gag atg gag aac   2064
Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

atc ttt cac tgc cgg ctg tct cag ggg agg aac ctg atg cag tgg gtc   2112
Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

ccg tcc ctg cag ctg ctg ctg cag cca cac tct ctg gag tcc ctc cac   2160
Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

tgc ttg tac gag act cgg aac aaa acg ttc ctg aca caa gtg atg gcc   2208
Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

cat ttc gaa gaa atg ggc atg tgt gta gaa aca gac atg gag ctc tta   2256
His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

gtg tgc act ttc tgc att aaa ttc agc cgc cac gtg aag aag ctt cag   2304
Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

ctg att gag ggc agg cag cac aga tca aca tgg agc ccc acc atg gta   2352
Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

gtc ctg ttc agg tgg gtc cca gtc aca gat gcc tat tgg cag att ctc   2400
Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

ttc tcc gtc ctc aag gtc acc aga aac ctg aag gag ctg gac cta agt   2448
Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

gga aac tcg ctg agc cac tct gca gtg aag agt ctt tgt aag acc ctg   2496
Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

aga cgc cct cgc tgc ctc ctg gag acc ctg cgg ttg gct ggc tgt ggc   2544
Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

ctc aca gct gag gac tgc aag gac ctt gcc ttt ggg ctg aga gcc aac   2592
Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

cag acc ctg acc gag ctg gac ctg agc ttc aat gtg ctc acg gat gct   2640
Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

gga gcc aaa cac ctt tgc cag aga ctg aga cag ccg agc tgc aag cta   2688
Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

cag cga ctg cag ctg gtc agc tgt ggc ctc acg tct gac tgc tgc cag   2736
Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910
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gac ctg gcc tct gtg ctt agt gcc agc ccc agc ctg aag gag cta gac   2784
Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

ctg cag cag aac aac ctg gat gac gtt ggc gtg cga ctg ctc tgt gag   2832
Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

ggg ctc agg cat cct gcc tgc aaa ctc ata cgc ctg aaa cca agt gtg   2880
Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Lys Pro Ser Val
945                 950                 955                 960

atg acc cct act gag ggc ctg gat acg gga gag atg agt aat agc aca   2928
Met Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser Thr
                965                 970                 975

tcc tca ctc aag cgg cag aga ctc gga tca gag agg gcg gct tcc cat   2976
Ser Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser His
            980                 985                 990

gtt gct cag gct aat ctc aaa ctc ctg gac gtg agc aag atc ttc cca   3024
Val Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe Pro
        995                 1000                1005

att gct gag att gca gag gaa agc tcc cca gag gta gta ccg gtg gaa   3072
Ile Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val Glu
   1010                1015                1020

ctc ttg tgc gtg cct tct cct gcc tct caa ggg gac ctg cat acg aag   3120
Leu Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr Lys
1025                1030                1035                1040

cct ttg ggg act gac gat gac ttc tgg ggc ccc acg ggg cct gtg gct   3168
Pro Leu Gly Thr Asp Asp Asp Phe Trp Gly Pro Thr Gly Pro Val Ala
                1045                1050                1055

act gag gta gtt gac aaa gaa aag aac ttg tac cga gtt cac ttc cct   3216
Thr Glu Val Val Asp Lys Glu Lys Asn Leu Tyr Arg Val His Phe Pro
            1060                1065                1070

gta gct ggc tcc tac cgc tgg ccc aac acg ggt ctc tgc ttt gtg atg   3264
Val Ala Gly Ser Tyr Arg Trp Pro Asn Thr Gly Leu Cys Phe Val Met
        1075                1080                1085

aga gaa gcg gtg acc gtt gag att gaa ttc tgt gtg tgg gac cag ttc   3312
Arg Glu Ala Val Thr Val Glu Ile Glu Phe Cys Val Trp Asp Gln Phe
    1090                1095                1100

ctg ggt gag atc aac cca cag cac agc tgg atg gtg gca ggg cct ctg   3360
Leu Gly Glu Ile Asn Pro Gln His Ser Trp Met Val Ala Gly Pro Leu
1105                1110                1115                1120

ctg gac atc aag gct gag cct gga gct gtg gaa gct gtg cac ctc cct   3408
Leu Asp Ile Lys Ala Glu Pro Gly Ala Val Glu Ala Val His Leu Pro
                1125                1130                1135

cac ttt gtg gct ctc caa ggg ggc cat gtg gac aca tcc ctg ttc caa   3456
His Phe Val Ala Leu Gln Gly Gly His Val Asp Thr Ser Leu Phe Gln
            1140                1145                1150

atg gcc cac ttt aaa gag gag ggg atg ctc ctg gag aag cca gcc agg   3504
Met Ala His Phe Lys Glu Glu Gly Met Leu Leu Glu Lys Pro Ala Arg
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       1155                1160                1165

gtg gag ctg cat cac ata gtt ctg gaa aac ccc agc ttc tcc ccc ttg   3552
Val Glu Leu His His Ile Val Leu Glu Asn Pro Ser Phe Ser Pro Leu
   1170                1175                1180

gga gtc ctc ctg aaa atg atc cat aat gcc ctg cgc ttc att ccc gtc   3600
Gly Val Leu Leu Lys Met Ile His Asn Ala Leu Arg Phe Ile Pro Val
1185                1190                1195                1200

acc tct gtg gtg ttg ctt tac cac cgc gtc cat cct gag gaa gtc acc   3648
Thr Ser Val Val Leu Leu Tyr His Arg Val His Pro Glu Glu Val Thr
               1205                1210                1215

ttc cac ctc tac ctg atc cca agt gac tgc tcc att cgg aag gcc ata   3696
Phe His Leu Tyr Leu Ile Pro Ser Asp Cys Ser Ile Arg Lys Ala Ile
          1220                1225                1230

gat gat cta gaa atg aaa ttc cag ttt gtg cga atc cac aag cca ccc   3744
Asp Asp Leu Glu Met Lys Phe Gln Phe Val Arg Ile His Lys Pro Pro
       1235                1240                1245

ccg ctg acc cca ctt tat atg ggc tgt cgt tac act gtg tct ggg tct   3792
Pro Leu Thr Pro Leu Tyr Met Gly Cys Arg Tyr Thr Val Ser Gly Ser
   1250                1255                1260

ggt tca ggg atg ctg gaa ata ctc ccc aag gaa ctg gag ctc tgc tat   3840
Gly Ser Gly Met Leu Glu Ile Leu Pro Lys Glu Leu Glu Leu Cys Tyr
1265                1270                1275                1280

cga agc cct gga gaa gac cag ctg ttc tcg gag ttc tac gtt ggc cac   3888
Arg Ser Pro Gly Glu Asp Gln Leu Phe Ser Glu Phe Tyr Val Gly His
               1285                1290                1295

ttg gga tca ggg atc agg ctg caa gtg aaa gac aag aaa gat gag act   3936
Leu Gly Ser Gly Ile Arg Leu Gln Val Lys Asp Lys Lys Asp Glu Thr
          1300                1305                1310

ctg gtg tgg gag gcc ttg gtg aaa cca gga gat ctc atg cct gca act   3984
Leu Val Trp Glu Ala Leu Val Lys Pro Gly Asp Leu Met Pro Ala Thr
       1315                1320                1325

act ctg atc cct cca gcc cgc ata gcc gta cct tca cct ctg gat gcc   4032
Thr Leu Ile Pro Pro Ala Arg Ile Ala Val Pro Ser Pro Leu Asp Ala
   1330                1335                1340

ccg cag ttg ctg cac ttt gtg gac cag tat cga gag cag ctg ata gcc   4080
Pro Gln Leu Leu His Phe Val Asp Gln Tyr Arg Glu Gln Leu Ile Ala
1345                1350                1355                1360

cga gtg aca tcg gtg gag gtt gtc ttg gac aaa ctg cat gga cag gtg   4128
Arg Val Thr Ser Val Glu Val Val Leu Asp Lys Leu His Gly Gln Val
               1365                1370                1375

ctg agc cag gag cag tac gag agg gtg ctg gct gag aac acg agg ccc   4176
Leu Ser Gln Glu Gln Tyr Glu Arg Val Leu Ala Glu Asn Thr Arg Pro
          1380                1385                1390

agc cag atg cgg aag ctg ttc agc ttg agc cag tcc tgg gac cgg aag   4224
Ser Gln Met Arg Lys Leu Phe Ser Leu Ser Gln Ser Trp Asp Arg Lys
       1395                1400                1405
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tgc aaa gat gga ctc tac caa gcc ctg aag gag acc cat cct cac ctc   4272
Cys Lys Asp Gly Leu Tyr Gln Ala Leu Lys Glu Thr His Pro His Leu
   1410                1415                1420

att atg gaa ctc tgg gag aag ggc agc aaa aag gga ctc ctg cca ctc   4320
Ile Met Glu Leu Trp Glu Lys Gly Ser Lys Lys Gly Leu Leu Pro Leu
1425                1430                1435                1440

agc agc tga                                                      4329
Ser Ser
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Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
            20                  25                  30

His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
        35                  40                  45

Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
    50                  55                  60

Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
65                  70                  75                  80

Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                85                  90                  95

Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
                165                 170                 175

Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240
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Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Gln Arg Pro His
        275                 280                 285

Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380

Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415

Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
    450                 455                 460

Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465                 470                 475                 480

Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495

Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575
```

```
Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605

Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625                 630                 635                 640

Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
            660                 665                 670

Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910
```

```
Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Lys Pro Ser Val
945                 950                 955                 960

Met Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser Thr
                965                 970                 975

Ser Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser His
            980                 985                 990

Val Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe Pro
        995                1000                1005

Ile Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val Glu
   1010                1015                1020

Leu Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr Lys
1025                1030                1035                1040

Pro Leu Gly Thr Asp Asp Asp Phe Trp Gly Pro Thr Gly Pro Val Ala
                1045                1050                1055

Thr Glu Val Val Asp Lys Glu Lys Asn Leu Tyr Arg Val His Phe Pro
            1060                1065                1070

Val Ala Gly Ser Tyr Arg Trp Pro Asn Thr Gly Leu Cys Phe Val Met
        1075                1080                1085

Arg Glu Ala Val Thr Val Glu Ile Glu Phe Cys Val Trp Asp Gln Phe
   1090                1095                1100

Leu Gly Glu Ile Asn Pro Gln His Ser Trp Met Val Ala Gly Pro Leu
1105                1110                1115                1120

Leu Asp Ile Lys Ala Glu Pro Gly Ala Val Glu Ala Val His Leu Pro
                1125                1130                1135

His Phe Val Ala Leu Gln Gly Gly His Val Asp Thr Ser Leu Phe Gln
            1140                1145                1150

Met Ala His Phe Lys Glu Glu Gly Met Leu Leu Glu Lys Pro Ala Arg
        1155                1160                1165

Val Glu Leu His His Ile Val Leu Glu Asn Pro Ser Phe Ser Pro Leu
   1170                1175                1180

Gly Val Leu Leu Lys Met Ile His Asn Ala Leu Arg Phe Ile Pro Val
1185                1190                1195                1200

Thr Ser Val Val Leu Leu Tyr His Arg Val His Pro Glu Glu Val Thr
                1205                1210                1215

Phe His Leu Tyr Leu Ile Pro Ser Asp Cys Ser Ile Arg Lys Ala Ile
            1220                1225                1230

Asp Asp Leu Glu Met Lys Phe Gln Phe Val Arg Ile His Lys Pro Pro
        1235                1240                1245
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Pro Leu Thr Pro Leu Tyr Met Gly Cys Arg Tyr Thr Val Ser Gly Ser
   1250                1255                1260

Gly Ser Gly Met Leu Glu Ile Leu Pro Lys Glu Leu Glu Leu Cys Tyr
1265                1270                1275                1280

Arg Ser Pro Gly Glu Asp Gln Leu Phe Ser Glu Phe Tyr Val Gly His
               1285                1290                1295

Leu Gly Ser Gly Ile Arg Leu Gln Val Lys Asp Lys Lys Asp Glu Thr
           1300                1305                1310

Leu Val Trp Glu Ala Leu Val Lys Pro Gly Asp Leu Met Pro Ala Thr
       1315                1320                1325

Thr Leu Ile Pro Pro Ala Arg Ile Ala Val Pro Ser Pro Leu Asp Ala
   1330                1335                1340

Pro Gln Leu Leu His Phe Val Asp Gln Tyr Arg Glu Gln Leu Ile Ala
1345                1350                1355                1360

Arg Val Thr Ser Val Glu Val Val Leu Asp Lys Leu His Gly Gln Val
               1365                1370                1375

Leu Ser Gln Glu Gln Tyr Glu Arg Val Leu Ala Glu Asn Thr Arg Pro
           1380                1385                1390

Ser Gln Met Arg Lys Leu Phe Ser Leu Ser Gln Ser Trp Asp Arg Lys
       1395                1400                1405

Cys Lys Asp Gly Leu Tyr Gln Ala Leu Lys Glu Thr His Pro His Leu
   1410                1415                1420

Ile Met Glu Leu Trp Glu Lys Gly Ser Lys Lys Gly Leu Leu Pro Leu
1425                1430                1435                1440

Ser Ser
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<210> 7
<211> 1487
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1293)

<400> 7

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atg atg aga cag agg cag agc cat tat tgt tcc gtg ctg ttc ctg agt   48
Met Met Arg Gln Arg Gln Ser His Tyr Cys Ser Val Leu Phe Leu Ser
  1               5                  10                  15

gtc aac tat ctg ggg ggg aca ttc cca gga gac att tgc tca gaa gag   96
Val Asn Tyr Leu Gly Gly Thr Phe Pro Gly Asp Ile Cys Ser Glu Glu
             20                  25                  30

aat caa ata gtt tcc tct tat gct tct aaa gtc tgt ttt gag atc gaa   144
Asn Gln Ile Val Ser Ser Tyr Ala Ser Lys Val Cys Phe Glu Ile Glu
         35                  40                  45
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```
gaa gat tat aaa aat cgt cag ttt ctg ggg cct gaa gga aat gtg gat   192
Glu Asp Tyr Lys Asn Arg Gln Phe Leu Gly Pro Glu Gly Asn Val Asp
     50                  55                  60

gtt gag ttg att gat aag agc aca aac aga tac agc gtt tgg ttc ccc   240
Val Glu Leu Ile Asp Lys Ser Thr Asn Arg Tyr Ser Val Trp Phe Pro
 65                  70                  75                  80

act gct ggc tgg tat ctg tgg tca gcc aca ggc ctc ggc ttc ctg gta   288
Thr Ala Gly Trp Tyr Leu Trp Ser Ala Thr Gly Leu Gly Phe Leu Val
                85                  90                  95

agg gat gag gtc aca gtg acg att gcg ttt ggt tcc tgg agt cag cac   336
Arg Asp Glu Val Thr Val Thr Ile Ala Phe Gly Ser Trp Ser Gln His
            100                 105                 110

ctg gcc ctg gac ctg cag cac cat gaa cag tgg ctg gtg ggc ggc ccc   384
Leu Ala Leu Asp Leu Gln His His Glu Gln Trp Leu Val Gly Gly Pro
        115                 120                 125

ttg ttt gat gtc act gca gag cca gag gag gct gtc gcc gaa atc cac   432
Leu Phe Asp Val Thr Ala Glu Pro Glu Glu Ala Val Ala Glu Ile His
    130                 135                 140

ctc ccc cac ttc atc tcc ctc caa ggt gag gtg gac gtc tcc tgg ttt   480
Leu Pro His Phe Ile Ser Leu Gln Gly Glu Val Asp Val Ser Trp Phe
145                 150                 155                 160

ctc gtt gcc cat ttt aag aat gaa ggg atg gtc ctg gag cat cca gcc   528
Leu Val Ala His Phe Lys Asn Glu Gly Met Val Leu Glu His Pro Ala
                165                 170                 175

cgg gtg gag cct ttc tat gct gtc ctg gaa agc ccc agc ttc tct ctg   576
Arg Val Glu Pro Phe Tyr Ala Val Leu Glu Ser Pro Ser Phe Ser Leu
            180                 185                 190

atg ggc atc ctg ctg cgg atc gcc agt ggg act cgc ctc tcc atc ccc   624
Met Gly Ile Leu Leu Arg Ile Ala Ser Gly Thr Arg Leu Ser Ile Pro
        195                 200                 205

atc act tcc aac aca ttg atc tat tat cac ccc cac ccc gaa gat att   672
Ile Thr Ser Asn Thr Leu Ile Tyr Tyr His Pro His Pro Glu Asp Ile
    210                 215                 220

aag ttc cac ttg tac ctt gtc ccc agc gac gcc ttg cta aca aag gcg   720
Lys Phe His Leu Tyr Leu Val Pro Ser Asp Ala Leu Leu Thr Lys Ala
225                 230                 235                 240

ata gat gat gag gaa gat cgc ttc cat ggt gtg cgc ctg cag act tcg   768
Ile Asp Asp Glu Glu Asp Arg Phe His Gly Val Arg Leu Gln Thr Ser
                245                 250                 255

ccc cca atg gaa ccc ctg aac ttt ggt tcc agt tat att gtg tct aat   816
Pro Pro Met Glu Pro Leu Asn Phe Gly Ser Ser Tyr Ile Val Ser Asn
            260                 265                 270

tct gct aac ctg aaa gta atg ccc aag gag ttg aaa ttg tcc tac agg   864
Ser Ala Asn Leu Lys Val Met Pro Lys Glu Leu Lys Leu Ser Tyr Arg
        275                 280                 285

agc cct gga gaa att cag cac ttc tca aaa ttc tat gct ggg cag atg   912
Ser Pro Gly Glu Ile Gln His Phe Ser Lys Phe Tyr Ala Gly Gln Met
```

```
    290                 295                 300

aag gaa ccc att caa ctt gag att act gaa aaa aga cat ggg act ttg   960
Lys Glu Pro Ile Gln Leu Glu Ile Thr Glu Lys Arg His Gly Thr Leu
305                 310                 315                 320

gtg tgg gat act gag gtg aag cca gtg gat ctc cag ctt gta gct gca   1008
Val Trp Asp Thr Glu Val Lys Pro Val Asp Leu Gln Leu Val Ala Ala
                325                 330                 335

tca gcc cct cct cct ttc tca ggt gca gcc ttt gtg aag gag aac cac   1056
Ser Ala Pro Pro Pro Phe Ser Gly Ala Ala Phe Val Lys Glu Asn His
            340                 345                 350

cgg caa ctc caa gcc agg atg ggg gac ctg aaa ggg gtg ctc gat gat   1104
Arg Gln Leu Gln Ala Arg Met Gly Asp Leu Lys Gly Val Leu Asp Asp
        355                 360                 365

ctc cag gac aat gag gtt ctt act gag aat gag aag gag ctg gtg gag   1152
Leu Gln Asp Asn Glu Val Leu Thr Glu Asn Glu Lys Glu Leu Val Glu
    370                 375                 380

cag gaa aag aca cgg cag agc aag aat gag gcc ttg ctg agc atg gtg   1200
Gln Glu Lys Thr Arg Gln Ser Lys Asn Glu Ala Leu Leu Ser Met Val
385                 390                 395                 400

gag aag aaa ggg gac ctg gcc ctg gac gtg ctc ttc aga agc att agt   1248
Glu Lys Lys Gly Asp Leu Ala Leu Asp Val Leu Phe Arg Ser Ile Ser
                405                 410                 415

gaa agg gac cct tac ctc gtg tcc tat ctt aga cag cag aat ttg       1293
Glu Arg Asp Pro Tyr Leu Val Ser Tyr Leu Arg Gln Gln Asn Leu
            420                 425                 430

taaaatgagt cagttaggta gtctggaaga gagaatccag cgttctcatt ggaaatggat 1353

aaacagaaat gtgatcattg atttcagtgt tcaagacaga agaagactgg gtaacatcta 1413

tcacacaggc tttcaggaca gacttgtaac ctggcatgta cctattgact gtatcctcat 1473

gcattttcct caag                                                   1487


<210> 8
<211> 431
<212> PRT
<213> Homo sapiens

<400> 8
Met Met Arg Gln Arg Gln Ser His Tyr Cys Ser Val Leu Phe Leu Ser
  1               5                  10                  15

Val Asn Tyr Leu Gly Gly Thr Phe Pro Gly Asp Ile Cys Ser Glu Glu
             20                  25                  30

Asn Gln Ile Val Ser Ser Tyr Ala Ser Lys Val Cys Phe Glu Ile Glu
         35                  40                  45

Glu Asp Tyr Lys Asn Arg Gln Phe Leu Gly Pro Glu Gly Asn Val Asp
     50                  55                  60

Val Glu Leu Ile Asp Lys Ser Thr Asn Arg Tyr Ser Val Trp Phe Pro
```

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65                  70                  75                  80

Thr Ala Gly Trp Tyr Leu Trp Ser Ala Thr Gly Leu Gly Phe Leu Val
                85                  90                  95

Arg Asp Glu Val Thr Val Thr Ile Ala Phe Gly Ser Trp Ser Gln His
            100                 105                 110

Leu Ala Leu Asp Leu Gln His His Glu Gln Trp Leu Val Gly Gly Pro
        115                 120                 125

Leu Phe Asp Val Thr Ala Glu Pro Glu Glu Ala Val Ala Glu Ile His
    130                 135                 140

Leu Pro His Phe Ile Ser Leu Gln Gly Glu Val Asp Val Ser Trp Phe
145                 150                 155                 160

Leu Val Ala His Phe Lys Asn Glu Gly Met Val Leu Glu His Pro Ala
                165                 170                 175

Arg Val Glu Pro Phe Tyr Ala Val Leu Glu Ser Pro Ser Phe Ser Leu
            180                 185                 190

Met Gly Ile Leu Leu Arg Ile Ala Ser Gly Thr Arg Leu Ser Ile Pro
        195                 200                 205

Ile Thr Ser Asn Thr Leu Ile Tyr Tyr His Pro His Pro Glu Asp Ile
    210                 215                 220

Lys Phe His Leu Tyr Leu Val Pro Ser Asp Ala Leu Leu Thr Lys Ala
225                 230                 235                 240

Ile Asp Asp Glu Glu Asp Arg Phe His Gly Val Arg Leu Gln Thr Ser
                245                 250                 255

Pro Pro Met Glu Pro Leu Asn Phe Gly Ser Ser Tyr Ile Val Ser Asn
            260                 265                 270

Ser Ala Asn Leu Lys Val Met Pro Lys Glu Leu Lys Leu Ser Tyr Arg
        275                 280                 285

Ser Pro Gly Glu Ile Gln His Phe Ser Lys Phe Tyr Ala Gly Gln Met
    290                 295                 300

Lys Glu Pro Ile Gln Leu Glu Ile Thr Glu Lys Arg His Gly Thr Leu
305                 310                 315                 320

Val Trp Asp Thr Glu Val Lys Pro Val Asp Leu Gln Leu Val Ala Ala
                325                 330                 335

Ser Ala Pro Pro Pro Phe Ser Gly Ala Ala Phe Val Lys Glu Asn His
            340                 345                 350

Arg Gln Leu Gln Ala Arg Met Gly Asp Leu Lys Gly Val Leu Asp Asp
        355                 360                 365

Leu Gln Asp Asn Glu Val Leu Thr Glu Asn Glu Lys Glu Leu Val Glu
    370                 375                 380

Gln Glu Lys Thr Arg Gln Ser Lys Asn Glu Ala Leu Leu Ser Met Val
385                 390                 395                 400
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Glu Lys Lys Gly Asp Leu Ala Leu Asp Val Leu Phe Arg Ser Ile Ser
                405                 410                 415

Glu Arg Asp Pro Tyr Leu Val Ser Tyr Leu Arg Gln Gln Asn Leu
            420                 425                 430


<210> 9
<211> 4556
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(4362)

<220>
<223> Description of Artificial Sequence: Synthetic
      Construct

<400> 9
atg gct ggc gga gcc tgg ggc cgc ctg gcc tgt tac ttg gag ttc ctg   48
Met Ala Gly Gly Ala Trp Gly Arg Leu Ala Cys Tyr Leu Glu Phe Leu
  1               5                  10                  15

aag aag gag gag ctg aag gag ttc cag ctt ctg ctc gcc aat aaa gcg   96
Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
             20                  25                  30

cac tcc agg agc tct tcg ggt gag aca ccc gct cag cca gag aag acg   144
His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
         35                  40                  45

agt ggc atg gag gtg gcc tcg tac ctg gtg gct cag tat ggg gag cag   192
Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
     50                  55                  60

cgg gcc tgg gac cta gcc ctc cat acc tgg gag cag atg ggg ctg agg   240
Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
 65                  70                  75                  80

tca ctg tgc gcc caa gcc cag gaa ggg gca ggc cac tct ccc tca ttc   288
Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                85                  90                  95

ccc tac agc cca agt gaa ccc cac ctg ggg tct ccc agc caa ccc acc   336
Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

tcc acc gca gtg cta atg ccc tgg atc cat gaa ttg ccg gcg ggg tgc   384
Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

acc cag ggc tca gag aga agg gtt ttg aga cag ctg cct gac aca tct   432
Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

gga cgc cgc tgg aga gaa atc tct gcc tca ctc ctc tac caa gct ctt   480
Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

cca agc tcc cca gac cat gag tct cca agc cag gag tca ccc aac gcc   528
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```
Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
                165                 170                 175

ccc aca tcc aca gca gtg ctg ggg agc tgg gga tcc cca cct cag ccc   576
Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

agc cta gca ccc aga gag cag gag gct cct ggg acc caa tgg cct ctg   624
Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

gat gaa acg tca gga att tac tac aca gaa atc aga gaa aga gag aga   672
Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

gag aaa tca gag aaa ggc agg ccc cca tgg gca gcg gtg gta gga acg   720
Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

ccc cca cag gcg cac acc agc cta cag ccc cac cac cac cca tgg gag   768
Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

cct tct gtg aga gag agc ctc tgt tcc aca tgg ccc tgg aaa aat gag   816
Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

gat ttt aac caa aaa ttc aca cag ctg cta ctt cta caa aga cct cac   864
Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
        275                 280                 285

ccc aga agc caa gat ccc ctg gtc aag aga agc tgg cct gat tat gtg   912
Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

gag gag aat cga gga cat tta att gag atc aga gac tta ttt ggc cca   960
Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

ggc ctg gat acc caa gaa cct cgc ata gtc ata ctg cag ggg gct gct  1008
Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

gga att ggg aag tca aca ctg gcc agg cag gtg aag gaa gcc tgg ggg  1056
Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

aga ggc cag ctg tat ggg gac cgc ttc cag cat gtc ttc tac ttc agc  1104
Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

tgc aga gag ctg gcc cag tcc aag gtg gtg agt ctc gct gag ctc atc  1152
Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380

gga aaa gat ggg aca gcc act ccg gct ccc att aga cag atc ctg tct  1200
Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

agg cca gag cgg ctg ctc ttc atc ctc gat ggt gta gat gag cca gga  1248
Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415
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tgg gtc ttg cag gag ccg agt tct gag ctc tgt ctg cac tgg agc cag   1296
Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

cca cag ccg gcg gat gca ctg ctg ggc agt ttg ctg ggg aaa act ata   1344
Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

ctt ccc gag gca tcc ttc ctg atc acg gct cgg acc aca gct ctg cag   1392
Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
    450                 455                 460

aac ctc att cct tct ttg gag cag gca cgt tgg gta gag gtc ctg ggg   1440
Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465                 470                 475                 480

ttc tct gag tcc agc agg aag gaa tat ttc tac aga tat ttc aca gat   1488
Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495

gaa agg caa gca att aga gcc ttt agg ttg gtc aaa tca aac aaa gag   1536
Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

ctc tgg gcc ctg tgt ctt gtg ccc tgg gtg tcc tgg ctg gcc tgc act   1584
Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

tgc ctg atg cag cag atg aag cgg aag gaa aaa ctc aca ctg act tcc   1632
Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

aag acc acc aca acc ctc tgt cta cat tac ctt gcc cag gct ctc caa   1680
Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

gct cag cca ttg gga ccc cag ctc aga gac ctc tgc tct ctg gct gct   1728
Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575

gag ggc atc tgg caa aaa aag acc ctt ttc agt cca gat gac ctc agg   1776
Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

aag cat ggg tta gat ggg gcc atc atc tcc acc ttc ttg aag atg ggt   1824
Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605

att ctt caa gag cac ccc atc cct ctg agc tac agc ttc att cac ctc   1872
Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

tgt ttc caa gag ttc ttt gca gca atg tcc tat gtc ttg gag gat gag   1920
Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625                 630                 635                 640

aag ggg aga ggt aaa cat tct aat tgc atc ata gat ttg gaa aag acg   1968
Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

cta gaa gca tat gga ata cat ggc ctg ttt ggg gca tca acc aca cgt   2016
Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
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            660                 665                 670

ttc cta ttg ggc ctg tta agt gat gag ggg gag aga gag atg gag aac   2064
Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

atc ttt cac tgc cgg ctg tct cag ggg agg aac ctg atg cag tgg gtc   2112
Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

ccg tcc ctg cag ctg ctg ctg cag cca cac tct ctg gag tcc ctc cac   2160
Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

tgc ttg tac gag act cgg aac aaa acg ttc ctg aca caa gtg atg gcc   2208
Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

cat ttc gaa gaa atg ggc atg tgt gta gaa aca gac atg gag ctc tta   2256
His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

gtg tgc act ttc tgc att aaa ttc agc cgc cac gtg aag aag ctt cag   2304
Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

ctg att gag ggc agg cag cac aga tca aca tgg agc ccc acc atg gta   2352
Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

gtc ctg ttc agg tgg gtc cca gtc aca gat gcc tat tgg cag att ctc   2400
Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

ttc tcc gtc ctc aag gtc acc aga aac ctg aag gag ctg gac cta agt   2448
Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

gga aac tcg ctg agc cac tct gca gtg aag agt ctt tgt aag acc ctg   2496
Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

aga cgc cct cgc tgc ctc ctg gag acc ctg cgg ttg gct ggc tgt ggc   2544
Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

ctc aca gct gag gac tgc aag gac ctt gcc ttt ggg ctg aga gcc aac   2592
Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

cag acc ctg acc gag ctg gac ctg agc ttc aat gtg ctc acg gat gct   2640
Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

gga gcc aaa cac ctt tgc cag aga ctg aga cag ccg agc tgc aag cta   2688
Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

cag cga ctg cag ctg gtc agc tgt ggc ctc acg tct gac tgc tgc cag   2736
Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910
```

```
gac ctg gcc tct gtg ctt agt gcc agc ccc agc ctg aag gag cta gac   2784
Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

ctg cag cag aac aac ctg gat gac gtt ggc gtg cga ctg ctc tgt gag   2832
Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

ggg ctc agg cat cct gcc tgc aaa ctc ata cgc ctg ggg ctg gac cag   2880
Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Gly Leu Asp Gln
945                 950                 955                 960

aca act ctg agt gat gag atg agg cag gaa ctg agg gcc ctg gag cag   2928
Thr Thr Leu Ser Asp Glu Met Arg Gln Glu Leu Arg Ala Leu Glu Gln
                965                 970                 975

gag aaa cct cag ctg ctc atc ttc agc aga cgg aaa cca agt gtg atg   2976
Glu Lys Pro Gln Leu Leu Ile Phe Ser Arg Arg Lys Pro Ser Val Met
            980                 985                 990

acc cct act gag ggc ctg gat acg gga gag atg agt aat agc aca tcc   3024
Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser Thr Ser
        995                1000                1005

tca ctc aag cgg cag aga ctc gga tca gag agg gcg gct tcc cat gtt   3072
Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser His Val
   1010                1015                1020

gct cag gct aat ctc aaa ctc ctg gac gtg agc aag atc ttc cca att   3120
Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe Pro Ile
1025                1030                1035                1040

gct gag att gca gag gaa agc tcc cca gag gta gta ccg gtg gaa ctc   3168
Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val Glu Leu
               1045                1050                1055

ttg tgc gtg cct tct cct gcc tct caa ggg gac ctg cat acg aag cct   3216
Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr Lys Pro
           1060                1065                1070

ttg ggg act gac gat gac ttt ctg ggg cct gaa gga aat gtg gat gtt   3264
Leu Gly Thr Asp Asp Asp Phe Leu Gly Pro Glu Gly Asn Val Asp Val
       1075                1080                1085

gag ttg att gat aag agc aca aac aga tac agc gtt tgg ttc ccc act   3312
Glu Leu Ile Asp Lys Ser Thr Asn Arg Tyr Ser Val Trp Phe Pro Thr
   1090                1095                1100

gct ggc tgg tat ctg tgg tca gcc aca ggc ctc ggc ttc ctg gta agg   3360
Ala Gly Trp Tyr Leu Trp Ser Ala Thr Gly Leu Gly Phe Leu Val Arg
1105                1110                1115                1120

gat gag gtc aca gtg acg att gcg ttt ggt tcc tgg agt cag cac ctg   3408
Asp Glu Val Thr Val Thr Ile Ala Phe Gly Ser Trp Ser Gln His Leu
               1125                1130                1135

gcc ctg gac ctg cag cac cat gaa cag tgg ctg gtg ggc ggc ccc ttg   3456
Ala Leu Asp Leu Gln His His Glu Gln Trp Leu Val Gly Gly Pro Leu
           1140                1145                1150

ttt gat gtc act gca gag cca gag gag gct gtc gcc gaa atc cac ctc   3504
Phe Asp Val Thr Ala Glu Pro Glu Glu Ala Val Ala Glu Ile His Leu
```

```
      1155                1160                1165

ccc cac ttc atc tcc ctc caa ggt gag gtg gac gtc tcc tgg ttt ctc   3552
Pro His Phe Ile Ser Leu Gln Gly Glu Val Asp Val Ser Trp Phe Leu
   1170                1175                1180

gtt gcc cat ttt aag aat gaa ggg atg gtc ctg gag cat cca gcc cgg   3600
Val Ala His Phe Lys Asn Glu Gly Met Val Leu Glu His Pro Ala Arg
1185                1190                1195                1200

gtg gag cct ttc tat gct gtc ctg gaa agc ccc agc ttc tct ctg atg   3648
Val Glu Pro Phe Tyr Ala Val Leu Glu Ser Pro Ser Phe Ser Leu Met
               1205                1210                1215

ggc atc ctg ctg cgg atc gcc agt ggg act cgc ctc tcc atc ccc atc   3696
Gly Ile Leu Leu Arg Ile Ala Ser Gly Thr Arg Leu Ser Ile Pro Ile
          1220                1225                1230

act tcc aac aca ttg atc tat tat cac ccc cac ccc gaa gat att aag   3744
Thr Ser Asn Thr Leu Ile Tyr Tyr His Pro His Pro Glu Asp Ile Lys
       1235                1240                1245

ttc cac ttg tac ctt gtc ccc agc gac gcc ttg cta aca aag gcg ata   3792
Phe His Leu Tyr Leu Val Pro Ser Asp Ala Leu Leu Thr Lys Ala Ile
   1250                1255                1260

gat gat gag gaa gat cgc ttc cat ggt gtg cgc ctg cag act tcg ccc   3840
Asp Asp Glu Glu Asp Arg Phe His Gly Val Arg Leu Gln Thr Ser Pro
1265                1270                1275                1280

cca atg gaa ccc ctg aac ttt ggt tcc agt tat att gtg tct aat tct   3888
Pro Met Glu Pro Leu Asn Phe Gly Ser Ser Tyr Ile Val Ser Asn Ser
               1285                1290                1295

gct aac ctg aaa gta atg ccc aag gag ttg aaa ttg tcc tac agg agc   3936
Ala Asn Leu Lys Val Met Pro Lys Glu Leu Lys Leu Ser Tyr Arg Ser
          1300                1305                1310

cct gga gaa att cag cac ttc tca aaa ttc tat gct ggg cag atg aag   3984
Pro Gly Glu Ile Gln His Phe Ser Lys Phe Tyr Ala Gly Gln Met Lys
       1315                1320                1325

gaa ccc att caa ctt gag att act gaa aaa aga cat ggg act ttg gtg   4032
Glu Pro Ile Gln Leu Glu Ile Thr Glu Lys Arg His Gly Thr Leu Val
   1330                1335                1340

tgg gat act gag gtg aag cca gtg gat ctc cag ctt gta gct gca tca   4080
Trp Asp Thr Glu Val Lys Pro Val Asp Leu Gln Leu Val Ala Ala Ser
1345                1350                1355                1360

gcc cct cct cct ttc tca ggt gca gcc ttt gtg aag gag aac cac cgg   4128
Ala Pro Pro Pro Phe Ser Gly Ala Ala Phe Val Lys Glu Asn His Arg
               1365                1370                1375

caa ctc caa gcc agg atg ggg gac ctg aaa ggg gtg ctc gat gat ctc   4176
Gln Leu Gln Ala Arg Met Gly Asp Leu Lys Gly Val Leu Asp Asp Leu
          1380                1385                1390

cag gac aat gag gtt ctt act gag aat gag aag gag ctg gtg gag cag   4224
Gln Asp Asn Glu Val Leu Thr Glu Asn Glu Lys Glu Leu Val Glu Gln
       1395                1400                1405
```

```
gaa aag aca cgg cag agc aag aat gag gcc ttg ctg agc atg gtg gag   4272
Glu Lys Thr Arg Gln Ser Lys Asn Glu Ala Leu Leu Ser Met Val Glu
   1410                1415                1420

aag aaa ggg gac ctg gcc ctg gac gtg ctc ttc aga agc att agt gaa   4320
Lys Lys Gly Asp Leu Ala Leu Asp Val Leu Phe Arg Ser Ile Ser Glu
1425               1430                1435                1440

agg gac cct tac ctc gtg tcc tat ctt aga cag cag aat ttg           4362
Arg Asp Pro Tyr Leu Val Ser Tyr Leu Arg Gln Gln Asn Leu
               1445                1450

taaaatgagt cagttaggta gtctggaaga gagaatccag cgttctcatt ggaaatggat 4422

aaacagaaat gtgatcattg atttcagtgt tcaagacaga agaagactgg gtaacatcta 4482

tcacacaggc tttcaggaca gacttgtaac ctggcatgta cctattgact gtatcctcat 4542

gcattttcct caag                                                   4556
```

<210> 10
<211> 1454
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic Construct

<400> 10

```
Met Ala Gly Gly Ala Trp Gly Arg Leu Ala Cys Tyr Leu Glu Phe Leu
  1               5                  10                  15

Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
             20                  25                  30

His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
         35                  40                  45

Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
     50                  55                  60

Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
 65                  70                  75                  80

Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                 85                  90                  95

Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
```

```
                165                 170                 175

Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
        275                 280                 285

Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380

Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415

Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
    450                 455                 460

Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465                 470                 475                 480

Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495
```

```
Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575

Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605

Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625                 630                 635                 640

Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
            660                 665                 670

Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830
```

```
Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910

Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Gly Leu Asp Gln
945                 950                 955                 960

Thr Thr Leu Ser Asp Glu Met Arg Gln Glu Leu Arg Ala Leu Glu Gln
                965                 970                 975

Glu Lys Pro Gln Leu Leu Ile Phe Ser Arg Arg Lys Pro Ser Val Met
            980                 985                 990

Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser Thr Ser
        995                1000                1005

Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser His Val
   1010                1015                1020

Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe Pro Ile
1025                1030                1035                1040

Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val Glu Leu
                1045                1050                1055

Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr Lys Pro
            1060                1065                1070

Leu Gly Thr Asp Asp Asp Phe Leu Gly Pro Glu Gly Asn Val Asp Val
        1075                1080                1085

Glu Leu Ile Asp Lys Ser Thr Asn Arg Tyr Ser Val Trp Phe Pro Thr
   1090                1095                1100

Ala Gly Trp Tyr Leu Trp Ser Ala Thr Gly Leu Gly Phe Leu Val Arg
1105                1110                1115                1120

Asp Glu Val Thr Val Thr Ile Ala Phe Gly Ser Trp Ser Gln His Leu
                1125                1130                1135

Ala Leu Asp Leu Gln His His Glu Gln Trp Leu Val Gly Gly Pro Leu
            1140                1145                1150

Phe Asp Val Thr Ala Glu Pro Glu Glu Ala Val Ala Glu Ile His Leu
        1155                1160                1165
```

```
Pro His Phe Ile Ser Leu Gln Gly Glu Val Asp Val Ser Trp Phe Leu
   1170                1175                1180

Val Ala His Phe Lys Asn Glu Gly Met Val Leu Glu His Pro Ala Arg
1185                1190                1195                1200

Val Glu Pro Phe Tyr Ala Val Leu Glu Ser Pro Ser Phe Ser Leu Met
               1205                1210                1215

Gly Ile Leu Leu Arg Ile Ala Ser Gly Thr Arg Leu Ser Ile Pro Ile
           1220                1225                1230

Thr Ser Asn Thr Leu Ile Tyr Tyr His Pro His Pro Glu Asp Ile Lys
       1235                1240                1245

Phe His Leu Tyr Leu Val Pro Ser Asp Ala Leu Leu Thr Lys Ala Ile
   1250                1255                1260

Asp Asp Glu Glu Asp Arg Phe His Gly Val Arg Leu Gln Thr Ser Pro
1265                1270                1275                1280

Pro Met Glu Pro Leu Asn Phe Gly Ser Ser Tyr Ile Val Ser Asn Ser
               1285                1290                1295

Ala Asn Leu Lys Val Met Pro Lys Glu Leu Lys Leu Ser Tyr Arg Ser
           1300                1305                1310

Pro Gly Glu Ile Gln His Phe Ser Lys Phe Tyr Ala Gly Gln Met Lys
       1315                1320                1325

Glu Pro Ile Gln Leu Glu Ile Thr Glu Lys Arg His Gly Thr Leu Val
   1330                1335                1340

Trp Asp Thr Glu Val Lys Pro Val Asp Leu Gln Leu Val Ala Ala Ser
1345                1350                1355                1360

Ala Pro Pro Pro Phe Ser Gly Ala Ala Phe Val Lys Glu Asn His Arg
               1365                1370                1375

Gln Leu Gln Ala Arg Met Gly Asp Leu Lys Gly Val Leu Asp Asp Leu
           1380                1385                1390

Gln Asp Asn Glu Val Leu Thr Glu Asn Glu Lys Glu Leu Val Glu Gln
       1395                1400                1405

Glu Lys Thr Arg Gln Ser Lys Asn Glu Ala Leu Leu Ser Met Val Glu
   1410                1415                1420

Lys Lys Gly Asp Leu Ala Leu Asp Val Leu Phe Arg Ser Ile Ser Glu
1425                1430                1435                1440

Arg Asp Pro Tyr Leu Val Ser Tyr Leu Arg Gln Gln Asn Leu
               1445                1450
```

<210> 11
<211> 4466
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS

<222> (1)..(4272)

<220>
<223> Description of Artificial Sequence: Synthetic Construct

<400> 11

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atg gct ggc gga gcc tgg ggc cgc ctg gcc tgt tac ttg gag ttc ctg   48
Met Ala Gly Gly Ala Trp Gly Arg Leu Ala Cys Tyr Leu Glu Phe Leu
  1               5                  10                  15

aag aag gag gag ctg aag gag ttc cag ctt ctg ctc gcc aat aaa gcg   96
Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
             20                  25                  30

cac tcc agg agc tct tcg ggt gag aca ccc gct cag cca gag aag acg   144
His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
         35                  40                  45

agt ggc atg gag gtg gcc tcg tac ctg gtg gct cag tat ggg gag cag   192
Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
     50                  55                  60

cgg gcc tgg gac cta gcc ctc cat acc tgg gag cag atg ggg ctg agg   240
Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
 65                  70                  75                  80

tca ctg tgc gcc caa gcc cag gaa ggg gca ggc cac tct ccc tca ttc   288
Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                 85                  90                  95

ccc tac agc cca agt gaa ccc cac ctg ggg tct ccc agc caa ccc acc   336
Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

tcc acc gca gtg cta atg ccc tgg atc cat gaa ttg ccg gcg ggg tgc   384
Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

acc cag ggc tca gag aga agg gtt ttg aga cag ctg cct gac aca tct   432
Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

gga cgc cgc tgg aga gaa atc tct gcc tca ctc ctc tac caa gct ctt   480
Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

cca agc tcc cca gac cat gag tct cca agc cag gag tca ccc aac gcc   528
Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
                165                 170                 175

ccc aca tcc aca gca gtg ctg ggg agc tgg gga tcc cca cct cag ccc   576
Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

agc cta gca ccc aga gag cag gag gct cct ggg acc caa tgg cct ctg   624
Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

gat gaa acg tca gga att tac tac aca gaa atc aga gaa aga gag aga   672
Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220
```

```
gag aaa tca gag aaa ggc agg ccc cca tgg gca gcg gtg gta gga acg   720
Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

ccc cca cag gcg cac acc agc cta cag ccc cac cac cac cca tgg gag   768
Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

cct tct gtg aga gag agc ctc tgt tcc aca tgg ccc tgg aaa aat gag   816
Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

gat ttt aac caa aaa ttc aca cag ctg cta ctt cta caa aga cct cac   864
Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
        275                 280                 285

ccc aga agc caa gat ccc ctg gtc aag aga agc tgg cct gat tat gtg   912
Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

gag gag aat cga gga cat tta att gag atc aga gac tta ttt ggc cca   960
Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

ggc ctg gat acc caa gaa cct cgc ata gtc ata ctg cag ggg gct gct  1008
Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

gga att ggg aag tca aca ctg gcc agg cag gtg aag gaa gcc tgg ggg  1056
Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

aga ggc cag ctg tat ggg gac cgc ttc cag cat gtc ttc tac ttc agc  1104
Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

tgc aga gag ctg gcc cag tcc aag gtg gtg agt ctc gct gag ctc atc  1152
Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380

gga aaa gat ggg aca gcc act ccg gct ccc att aga cag atc ctg tct  1200
Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

agg cca gag cgg ctg ctc ttc atc ctc gat ggt gta gat gag cca gga  1248
Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415

tgg gtc ttg cag gag ccg agt tct gag ctc tgt ctg cac tgg agc cag  1296
Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

cca cag ccg gcg gat gca ctg ctg ggc agt ttg ctg ggg aaa act ata  1344
Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

ctt ccc gag gca tcc ttc ctg atc acg gct cgg acc aca gct ctg cag  1392
Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
    450                 455                 460

aac ctc att cct tct ttg gag cag gca cgt tgg gta gag gtc ctg ggg  1440
Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
```

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465                 470                 475                 480

ttc tct gag tcc agc agg aag gaa tat ttc tac aga tat ttc aca gat   1488
Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495

gaa agg caa gca att aga gcc ttt agg ttg gtc aaa tca aac aaa gag   1536
Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

ctc tgg gcc ctg tgt ctt gtg ccc tgg gtg tcc tgg ctg gcc tgc act   1584
Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

tgc ctg atg cag cag atg aag cgg aag gaa aaa ctc aca ctg act tcc   1632
Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

aag acc acc aca acc ctc tgt cta cat tac ctt gcc cag gct ctc caa   1680
Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

gct cag cca ttg gga ccc cag ctc aga gac ctc tgc tct ctg gct gct   1728
Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575

gag ggc atc tgg caa aaa aag acc ctt ttc agt cca gat gac ctc agg   1776
Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

aag cat ggg tta gat ggg gcc atc atc tcc acc ttc ttg aag atg ggt   1824
Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605

att ctt caa gag cac ccc atc cct ctg agc tac agc ttc att cac ctc   1872
Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

tgt ttc caa gag ttc ttt gca gca atg tcc tat gtc ttg gag gat gag   1920
Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625                 630                 635                 640

aag ggg aga ggt aaa cat tct aat tgc atc ata gat ttg gaa aag acg   1968
Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

cta gaa gca tat gga ata cat ggc ctg ttt ggg gca tca acc aca cgt   2016
Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
            660                 665                 670

ttc cta ttg ggc ctg tta agt gat gag ggg gag aga gag atg gag aac   2064
Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

atc ttt cac tgc cgg ctg tct cag ggg agg aac ctg atg cag tgg gtc   2112
Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

ccg tcc ctg cag ctg ctg ctg cag cca cac tct ctg gag tcc ctc cac   2160
Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720
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```
tgc ttg tac gag act cgg aac aaa acg ttc ctg aca caa gtg atg gcc   2208
Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

cat ttc gaa gaa atg ggc atg tgt gta gaa aca gac atg gag ctc tta   2256
His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

gtg tgc act ttc tgc att aaa ttc agc cgc cac gtg aag aag ctt cag   2304
Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

ctg att gag ggc agg cag cac aga tca aca tgg agc ccc acc atg gta   2352
Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

gtc ctg ttc agg tgg gtc cca gtc aca gat gcc tat tgg cag att ctc   2400
Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

ttc tcc gtc ctc aag gtc acc aga aac ctg aag gag ctg gac cta agt   2448
Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

gga aac tcg ctg agc cac tct gca gtg aag agt ctt tgt aag acc ctg   2496
Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

aga cgc cct cgc tgc ctc ctg gag acc ctg cgg ttg gct ggc tgt ggc   2544
Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

ctc aca gct gag gac tgc aag gac ctt gcc ttt ggg ctg aga gcc aac   2592
Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

cag acc ctg acc gag ctg gac ctg agc ttc aat gtg ctc acg gat gct   2640
Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

gga gcc aaa cac ctt tgc cag aga ctg aga cag ccg agc tgc aag cta   2688
Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

cag cga ctg cag ctg gtc agc tgt ggc ctc acg tct gac tgc tgc cag   2736
Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910

gac ctg gcc tct gtg ctt agt gcc agc ccc agc ctg aag gag cta gac   2784
Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

ctg cag cag aac aac ctg gat gac gtt ggc gtg cga ctg ctc tgt gag   2832
Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940

ggg ctc agg cat cct gcc tgc aaa ctc ata cgc ctg ggg aaa cca agt   2880
Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Gly Lys Pro Ser
945                 950                 955                 960

gtg atg acc cct act gag ggc ctg gat acg gga gag atg agt aat agc   2928
Val Met Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser
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                965                 970                 975

aca tcc tca ctc aag cgg cag aga ctc gga tca gag agg gcg gct tcc   2976
Thr Ser Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser
            980                 985                 990

cat gtt gct cag gct aat ctc aaa ctc ctg gac gtg agc aag atc ttc   3024
His Val Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe
        995                1000                1005

cca att gct gag att gca gag gaa agc tcc cca gag gta gta ccg gtg   3072
Pro Ile Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val
   1010                1015                1020

gaa ctc ttg tgc gtg cct tct cct gcc tct caa ggg gac ctg cat acg   3120
Glu Leu Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr
1025                1030                1035                1040

aag cct ttg ggg act gac gat gac ttt ctg ggg cct gaa gga aat gtg   3168
Lys Pro Leu Gly Thr Asp Asp Asp Phe Leu Gly Pro Glu Gly Asn Val
                1045                1050                1055

gat gtt gag ttg att gat aag agc aca aac aga tac agc gtt tgg ttc   3216
Asp Val Glu Leu Ile Asp Lys Ser Thr Asn Arg Tyr Ser Val Trp Phe
           1060                1065                1070

ccc act gct ggc tgg tat ctg tgg tca gcc aca ggc ctc ggc ttc ctg   3264
Pro Thr Ala Gly Trp Tyr Leu Trp Ser Ala Thr Gly Leu Gly Phe Leu
       1075                1080                1085

gta agg gat gag gtc aca gtg acg att gcg ttt ggt tcc tgg agt cag   3312
Val Arg Asp Glu Val Thr Val Thr Ile Ala Phe Gly Ser Trp Ser Gln
   1090                1095                1100

cac ctg gcc ctg gac ctg cag cac cat gaa cag tgg ctg gtg ggc ggc   3360
His Leu Ala Leu Asp Leu Gln His His Glu Gln Trp Leu Val Gly Gly
1105                1110                1115                1120

ccc ttg ttt gat gtc act gca gag cca gag gag gct gtc gcc gaa atc   3408
Pro Leu Phe Asp Val Thr Ala Glu Pro Glu Glu Ala Val Ala Glu Ile
                1125                1130                1135

cac ctc ccc cac ttc atc tcc ctc caa ggt gag gtg gac gtc tcc tgg   3456
His Leu Pro His Phe Ile Ser Leu Gln Gly Glu Val Asp Val Ser Trp
           1140                1145                1150

ttt ctc gtt gcc cat ttt aag aat gaa ggg atg gtc ctg gag cat cca   3504
Phe Leu Val Ala His Phe Lys Asn Glu Gly Met Val Leu Glu His Pro
       1155                1160                1165

gcc cgg gtg gag cct ttc tat gct gtc ctg gaa agc ccc agc ttc tct   3552
Ala Arg Val Glu Pro Phe Tyr Ala Val Leu Glu Ser Pro Ser Phe Ser
   1170                1175                1180

ctg atg ggc atc ctg ctg cgg atc gcc agt ggg act cgc ctc tcc atc   3600
Leu Met Gly Ile Leu Leu Arg Ile Ala Ser Gly Thr Arg Leu Ser Ile
1185                1190                1195                1200

ccc atc act tcc aac aca ttg atc tat tat cac ccc cac ccc gaa gat   3648
Pro Ile Thr Ser Asn Thr Leu Ile Tyr Tyr His Pro His Pro Glu Asp
                1205                1210                1215
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att aag ttc cac ttg tac ctt gtc ccc agc gac gcc ttg cta aca aag   3696
Ile Lys Phe His Leu Tyr Leu Val Pro Ser Asp Ala Leu Leu Thr Lys
          1220                1225                1230

gcg ata gat gat gag gaa gat cgc ttc cat ggt gtg cgc ctg cag act   3744
Ala Ile Asp Asp Glu Glu Asp Arg Phe His Gly Val Arg Leu Gln Thr
       1235                1240                1245

tcg ccc cca atg gaa ccc ctg aac ttt ggt tcc agt tat att gtg tct   3792
Ser Pro Pro Met Glu Pro Leu Asn Phe Gly Ser Ser Tyr Ile Val Ser
   1250                1255                1260

aat tct gct aac ctg aaa gta atg ccc aag gag ttg aaa ttg tcc tac   3840
Asn Ser Ala Asn Leu Lys Val Met Pro Lys Glu Leu Lys Leu Ser Tyr
1265                1270                1275                1280

agg agc cct gga gaa att cag cac ttc tca aaa ttc tat gct ggg cag   3888
Arg Ser Pro Gly Glu Ile Gln His Phe Ser Lys Phe Tyr Ala Gly Gln
             1285                1290                1295

atg aag gaa ccc att caa ctt gag att act gaa aaa aga cat ggg act   3936
Met Lys Glu Pro Ile Gln Leu Glu Ile Thr Glu Lys Arg His Gly Thr
          1300                1305                1310

ttg gtg tgg gat act gag gtg aag cca gtg gat ctc cag ctt gta gct   3984
Leu Val Trp Asp Thr Glu Val Lys Pro Val Asp Leu Gln Leu Val Ala
       1315                1320                1325

gca tca gcc cct cct cct ttc tca ggt gca gcc ttt gtg aag gag aac   4032
Ala Ser Ala Pro Pro Pro Phe Ser Gly Ala Ala Phe Val Lys Glu Asn
   1330                1335                1340

cac cgg caa ctc caa gcc agg atg ggg gac ctg aaa ggg gtg ctc gat   4080
His Arg Gln Leu Gln Ala Arg Met Gly Asp Leu Lys Gly Val Leu Asp
1345                1350                1355                1360

gat ctc cag gac aat gag gtt ctt act gag aat gag aag gag ctg gtg   4128
Asp Leu Gln Asp Asn Glu Val Leu Thr Glu Asn Glu Lys Glu Leu Val
             1365                1370                1375

gag cag gaa aag aca cgg cag agc aag aat gag gcc ttg ctg agc atg   4176
Glu Gln Glu Lys Thr Arg Gln Ser Lys Asn Glu Ala Leu Leu Ser Met
          1380                1385                1390

gtg gag aag aaa ggg gac ctg gcc ctg gac gtg ctc ttc aga agc att   4224
Val Glu Lys Lys Gly Asp Leu Ala Leu Asp Val Leu Phe Arg Ser Ile
       1395                1400                1405

agt gaa agg gac cct tac ctc gtg tcc tat ctt aga cag cag aat ttg   4272
Ser Glu Arg Asp Pro Tyr Leu Val Ser Tyr Leu Arg Gln Gln Asn Leu
   1410                1415                1420

taaaatgagt cagttaggta gtctggaaga gagaatccag cgttctcatt ggaaatggat 4332

aaacagaaat gtgatcattg atttcagtgt tcaagacaga agaagactgg gtaacatcta 4392

tcacacaggc tttcaggaca gacttgtaac ctggcatgta cctattgact gtatcctcat 4452

gcattttcct caag                                                   4466
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<210> 12

<211> 1424
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

Construct

<400> 12

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Met Ala Gly Gly Ala Trp Gly Arg Leu Ala Cys Tyr Leu Glu Phe Leu
  1               5                  10                  15

Lys Lys Glu Glu Leu Lys Glu Phe Gln Leu Leu Leu Ala Asn Lys Ala
             20                  25                  30

His Ser Arg Ser Ser Ser Gly Glu Thr Pro Ala Gln Pro Glu Lys Thr
         35                  40                  45

Ser Gly Met Glu Val Ala Ser Tyr Leu Val Ala Gln Tyr Gly Glu Gln
     50                  55                  60

Arg Ala Trp Asp Leu Ala Leu His Thr Trp Glu Gln Met Gly Leu Arg
 65                  70                  75                  80

Ser Leu Cys Ala Gln Ala Gln Glu Gly Ala Gly His Ser Pro Ser Phe
                 85                  90                  95

Pro Tyr Ser Pro Ser Glu Pro His Leu Gly Ser Pro Ser Gln Pro Thr
            100                 105                 110

Ser Thr Ala Val Leu Met Pro Trp Ile His Glu Leu Pro Ala Gly Cys
        115                 120                 125

Thr Gln Gly Ser Glu Arg Arg Val Leu Arg Gln Leu Pro Asp Thr Ser
    130                 135                 140

Gly Arg Arg Trp Arg Glu Ile Ser Ala Ser Leu Leu Tyr Gln Ala Leu
145                 150                 155                 160

Pro Ser Ser Pro Asp His Glu Ser Pro Ser Gln Glu Ser Pro Asn Ala
                165                 170                 175

Pro Thr Ser Thr Ala Val Leu Gly Ser Trp Gly Ser Pro Pro Gln Pro
            180                 185                 190

Ser Leu Ala Pro Arg Glu Gln Glu Ala Pro Gly Thr Gln Trp Pro Leu
        195                 200                 205

Asp Glu Thr Ser Gly Ile Tyr Tyr Thr Glu Ile Arg Glu Arg Glu Arg
    210                 215                 220

Glu Lys Ser Glu Lys Gly Arg Pro Pro Trp Ala Ala Val Val Gly Thr
225                 230                 235                 240

Pro Pro Gln Ala His Thr Ser Leu Gln Pro His His His Pro Trp Glu
                245                 250                 255

Pro Ser Val Arg Glu Ser Leu Cys Ser Thr Trp Pro Trp Lys Asn Glu
            260                 265                 270

Asp Phe Asn Gln Lys Phe Thr Gln Leu Leu Leu Leu Gln Arg Pro His
```

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        275                 280                 285

Pro Arg Ser Gln Asp Pro Leu Val Lys Arg Ser Trp Pro Asp Tyr Val
    290                 295                 300

Glu Glu Asn Arg Gly His Leu Ile Glu Ile Arg Asp Leu Phe Gly Pro
305                 310                 315                 320

Gly Leu Asp Thr Gln Glu Pro Arg Ile Val Ile Leu Gln Gly Ala Ala
                325                 330                 335

Gly Ile Gly Lys Ser Thr Leu Ala Arg Gln Val Lys Glu Ala Trp Gly
            340                 345                 350

Arg Gly Gln Leu Tyr Gly Asp Arg Phe Gln His Val Phe Tyr Phe Ser
        355                 360                 365

Cys Arg Glu Leu Ala Gln Ser Lys Val Val Ser Leu Ala Glu Leu Ile
    370                 375                 380

Gly Lys Asp Gly Thr Ala Thr Pro Ala Pro Ile Arg Gln Ile Leu Ser
385                 390                 395                 400

Arg Pro Glu Arg Leu Leu Phe Ile Leu Asp Gly Val Asp Glu Pro Gly
                405                 410                 415

Trp Val Leu Gln Glu Pro Ser Ser Glu Leu Cys Leu His Trp Ser Gln
            420                 425                 430

Pro Gln Pro Ala Asp Ala Leu Leu Gly Ser Leu Leu Gly Lys Thr Ile
        435                 440                 445

Leu Pro Glu Ala Ser Phe Leu Ile Thr Ala Arg Thr Thr Ala Leu Gln
    450                 455                 460

Asn Leu Ile Pro Ser Leu Glu Gln Ala Arg Trp Val Glu Val Leu Gly
465                 470                 475                 480

Phe Ser Glu Ser Ser Arg Lys Glu Tyr Phe Tyr Arg Tyr Phe Thr Asp
                485                 490                 495

Glu Arg Gln Ala Ile Arg Ala Phe Arg Leu Val Lys Ser Asn Lys Glu
            500                 505                 510

Leu Trp Ala Leu Cys Leu Val Pro Trp Val Ser Trp Leu Ala Cys Thr
        515                 520                 525

Cys Leu Met Gln Gln Met Lys Arg Lys Glu Lys Leu Thr Leu Thr Ser
    530                 535                 540

Lys Thr Thr Thr Thr Leu Cys Leu His Tyr Leu Ala Gln Ala Leu Gln
545                 550                 555                 560

Ala Gln Pro Leu Gly Pro Gln Leu Arg Asp Leu Cys Ser Leu Ala Ala
                565                 570                 575

Glu Gly Ile Trp Gln Lys Lys Thr Leu Phe Ser Pro Asp Asp Leu Arg
            580                 585                 590

Lys His Gly Leu Asp Gly Ala Ile Ile Ser Thr Phe Leu Lys Met Gly
        595                 600                 605
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```
Ile Leu Gln Glu His Pro Ile Pro Leu Ser Tyr Ser Phe Ile His Leu
    610                 615                 620

Cys Phe Gln Glu Phe Phe Ala Ala Met Ser Tyr Val Leu Glu Asp Glu
625                 630                 635                 640

Lys Gly Arg Gly Lys His Ser Asn Cys Ile Ile Asp Leu Glu Lys Thr
                645                 650                 655

Leu Glu Ala Tyr Gly Ile His Gly Leu Phe Gly Ala Ser Thr Thr Arg
            660                 665                 670

Phe Leu Leu Gly Leu Leu Ser Asp Glu Gly Glu Arg Glu Met Glu Asn
        675                 680                 685

Ile Phe His Cys Arg Leu Ser Gln Gly Arg Asn Leu Met Gln Trp Val
    690                 695                 700

Pro Ser Leu Gln Leu Leu Leu Gln Pro His Ser Leu Glu Ser Leu His
705                 710                 715                 720

Cys Leu Tyr Glu Thr Arg Asn Lys Thr Phe Leu Thr Gln Val Met Ala
                725                 730                 735

His Phe Glu Glu Met Gly Met Cys Val Glu Thr Asp Met Glu Leu Leu
            740                 745                 750

Val Cys Thr Phe Cys Ile Lys Phe Ser Arg His Val Lys Lys Leu Gln
        755                 760                 765

Leu Ile Glu Gly Arg Gln His Arg Ser Thr Trp Ser Pro Thr Met Val
    770                 775                 780

Val Leu Phe Arg Trp Val Pro Val Thr Asp Ala Tyr Trp Gln Ile Leu
785                 790                 795                 800

Phe Ser Val Leu Lys Val Thr Arg Asn Leu Lys Glu Leu Asp Leu Ser
                805                 810                 815

Gly Asn Ser Leu Ser His Ser Ala Val Lys Ser Leu Cys Lys Thr Leu
            820                 825                 830

Arg Arg Pro Arg Cys Leu Leu Glu Thr Leu Arg Leu Ala Gly Cys Gly
        835                 840                 845

Leu Thr Ala Glu Asp Cys Lys Asp Leu Ala Phe Gly Leu Arg Ala Asn
    850                 855                 860

Gln Thr Leu Thr Glu Leu Asp Leu Ser Phe Asn Val Leu Thr Asp Ala
865                 870                 875                 880

Gly Ala Lys His Leu Cys Gln Arg Leu Arg Gln Pro Ser Cys Lys Leu
                885                 890                 895

Gln Arg Leu Gln Leu Val Ser Cys Gly Leu Thr Ser Asp Cys Cys Gln
            900                 905                 910

Asp Leu Ala Ser Val Leu Ser Ala Ser Pro Ser Leu Lys Glu Leu Asp
        915                 920                 925

Leu Gln Gln Asn Asn Leu Asp Asp Val Gly Val Arg Leu Leu Cys Glu
    930                 935                 940
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```
Gly Leu Arg His Pro Ala Cys Lys Leu Ile Arg Leu Gly Lys Pro Ser
945                 950                 955                 960

Val Met Thr Pro Thr Glu Gly Leu Asp Thr Gly Glu Met Ser Asn Ser
                965                 970                 975

Thr Ser Ser Leu Lys Arg Gln Arg Leu Gly Ser Glu Arg Ala Ala Ser
            980                 985                 990

His Val Ala Gln Ala Asn Leu Lys Leu Leu Asp Val Ser Lys Ile Phe
        995                1000                1005

Pro Ile Ala Glu Ile Ala Glu Glu Ser Ser Pro Glu Val Val Pro Val
   1010                1015                1020

Glu Leu Leu Cys Val Pro Ser Pro Ala Ser Gln Gly Asp Leu His Thr
1025                1030                1035                1040

Lys Pro Leu Gly Thr Asp Asp Asp Phe Leu Gly Pro Glu Gly Asn Val
                1045                1050                1055

Asp Val Glu Leu Ile Asp Lys Ser Thr Asn Arg Tyr Ser Val Trp Phe
            1060                1065                1070

Pro Thr Ala Gly Trp Tyr Leu Trp Ser Ala Thr Gly Leu Gly Phe Leu
        1075                1080                1085

Val Arg Asp Glu Val Thr Val Thr Ile Ala Phe Gly Ser Trp Ser Gln
   1090                1095                1100

His Leu Ala Leu Asp Leu Gln His His Glu Gln Trp Leu Val Gly Gly
1105                1110                1115                1120

Pro Leu Phe Asp Val Thr Ala Glu Pro Glu Glu Ala Val Ala Glu Ile
                1125                1130                1135

His Leu Pro His Phe Ile Ser Leu Gln Gly Glu Val Asp Val Ser Trp
            1140                1145                1150

Phe Leu Val Ala His Phe Lys Asn Glu Gly Met Val Leu Glu His Pro
        1155                1160                1165

Ala Arg Val Glu Pro Phe Tyr Ala Val Leu Glu Ser Pro Ser Phe Ser
   1170                1175                1180

Leu Met Gly Ile Leu Leu Arg Ile Ala Ser Gly Thr Arg Leu Ser Ile
1185                1190                1195                1200

Pro Ile Thr Ser Asn Thr Leu Ile Tyr Tyr His Pro His Pro Glu Asp
                1205                1210                1215

Ile Lys Phe His Leu Tyr Leu Val Pro Ser Asp Ala Leu Leu Thr Lys
            1220                1225                1230

Ala Ile Asp Asp Glu Glu Asp Arg Phe His Gly Val Arg Leu Gln Thr
        1235                1240                1245

Ser Pro Pro Met Glu Pro Leu Asn Phe Gly Ser Ser Tyr Ile Val Ser
   1250                1255                1260

Asn Ser Ala Asn Leu Lys Val Met Pro Lys Glu Leu Lys Leu Ser Tyr
1265                1270                1275                1280
```

Arg Ser Pro Gly Glu Ile Gln His Phe Ser Lys Phe Tyr Ala Gly Gln
1285 1290 1295

Met Lys Glu Pro Ile Gln Leu Glu Ile Thr Glu Lys Arg His Gly Thr
1300 1305 1310

Leu Val Trp Asp Thr Glu Val Lys Pro Val Asp Leu Gln Leu Val Ala
1315 1320 1325

Ala Ser Ala Pro Pro Pro Phe Ser Gly Ala Ala Phe Val Lys Glu Asn
1330 1335 1340

His Arg Gln Leu Gln Ala Arg Met Gly Asp Leu Lys Gly Val Leu Asp
1345 1350 1355 1360

Asp Leu Gln Asp Asn Glu Val Leu Thr Glu Asn Glu Lys Glu Leu Val
1365 1370 1375

Glu Gln Glu Lys Thr Arg Gln Ser Lys Asn Glu Ala Leu Leu Ser Met
1380 1385 1390

Val Glu Lys Lys Gly Asp Leu Ala Leu Asp Val Leu Phe Arg Ser Ile
1395 1400 1405

Ser Glu Arg Asp Pro Tyr Leu Val Ser Tyr Leu Arg Gln Gln Asn Leu
1410 1415 1420

<210> 13
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 13
ccgaattcac catggctggc ggagcctggg gc 32

<210> 14
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 14
ccgctcgagt caacagaggg ttgtggtggt cttg 34

<210> 15
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 15
cccgaattcg aacctcgcat agtcatactg c 31

<210> 16
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 16
gtcccacaac agaattcaat ctcaacggtc 30

<210> 17
<211> 21
<212> DNA
<213> Homo sapiens

<400> 17
tgtgatgaga gaagcggtga c 21

<210> 18
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 18
ccgctcgagc aaagaagggt cagccaaagc 30